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# Soybean Genetics Newsletter



MAT'L AGRIC, LITELARY

Volume 11

April 1984

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Agricultural Research Service-USDA

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#### TABLE OF CONTENTS

		Page
I.	FOREWORD	1
II.	ANNOUNCEMENT	2
III.	SOYBEAN GENETICS COMMITTEE REPORT	3
IV.	SPECIAL REPORTS	
	Rhizobium japonicum strains maintained in culture collections throughout the world. R. G. Palmer	9
	Germplasm Advisory Committee. R. L. Nelson	14
	Germplasm Collection Report. R. L. Bernard, G. A. Juvick and R. L. Nelson	15
V.	RESEARCH NOTES	
	Australia	
	Wild perennial <i>Glycine</i> species as genetic resources for soybean improvement. A. H. D. Brown, J. E. Grant, J. J. Burdon and J. P. Grace	17
	India	
	Effects of sowing date and decapitation on green soybeans. S. C. Sarmah and A. K. Choudhury	21
	Effect of varieties and date of sowing on the growth and yield of soybean. S. C. Sarmah and A. K. Choudhury	24
	Effect of varieties and population densities on the growth and yield of soybean. S. C. Sarmah and A. K. Choudhury	27
	Effect of varieties and plant population on grain yield and two yield attributes of soybean. S. C. Sarmah and	
	M. M. Kalita	29
	Performance of winter soybean under varying levels of irrigation. S. C. Sarmah, S. Ahmed and A. K. Pathak	31
	Effect of dates of planting on five soybean varieties. S. C. Sarmah, M. M. Kalita and N. N. Kakati	34
	Phenotypic stability of soybean genotypes for field germination. P. Talukdar and B. K. Konwar	38
	Potential of an off-season soybean nursery. S. S. Banga, S. K. Banga, R. Prakash and J. B. Tomar	42
	Mutation breeding research in soybean in India. R. Prakash, H. B. P. Trivedi, V. Kerketta and M. F. Haque	43
	Induced parthenogenesis in soybean. S. K. Banga, S. S. Banga and M. Srivastava	45

	Breeding for resistance to yellow mosaic virus through inter- specific hybridization in soybean. H. H. Ram, K. Singh, Pushpendra, and V. D. Verma	46
	Japan	
	Effect of temperature on fasciation characters in fasciated soybean. W. Wongyai, T. Furuya and S. Matsumoto	49
	Poland	
	Identification and evaluation for mutation of agricultural characters in soybean. H. Skorupska	53
	United States	
100	Evaluation of soybean germplasm for stress tolerance and biological efficiency. V. Sapra, C. C. Tiwari, P. E. Igbokwe, S. Edung, L. Russel, B. T. Singh, M. Rangappa, P. S. Benepal, R. P. Pacumbaba, R. B. Dadson, J. Joshi, L. Murphy, D. S. Bhagsari, M. Floyd, R. Garner, S. Mookherji, M. R. Reddy and J. R. Allen	61
	In vitro fertilization of perennial soybean species. J. J. Silvoy, E. A. Rupert and E. R. Shipe	80
	Instability of virulence characters of soybean mosaic virus strains after seed transmission. J. Fletcher and R. M. Goodman	83
	The genus <i>Glycine</i> subgenus <i>Glycine</i> germplasm collection.  T. Hymowitz and R. J. Singh	86
	Hybridization in genus <i>Glycine</i> subgenus <i>Glycine</i> . R. J. Singh and T. Hymowitz	86
	A soybean x <i>Glycine tomentella</i> hybrid: Progress and problems. T. Hymowitz and R. J. Singh	90
	Superoxide dismutase (SOD) in soybean. J. D. Griffin and R. G. Palmer	91
	Inheritance of a miniature mutant in soybean. X. Delannay and R. G. Palmer	92
	Genetic studies with T263. R. G. Palmer	94
	Linkage group 12. R. G. Palmer, S. L. Broich and X. Delannay	97
	Molecular analysis of organelle genomes of a cytoplasmically inherited mutant of soybean. R. C. Shoemaker, R. G. Palmer and A. G. Atherly	100
	Is the ${\it ms}_4$ male-sterile mutant partially fertile? R. Graybosch and R. G. Palmer	102
	Evaluation of <i>Glycine soja</i> from The People's Republic of China and the USSR. R. G. Palmer and K. E. Newhouse	105
	A possible interaction between $cyt-Y_3$ and $y_{20}-k_2$ . R. C. Shoemaker and R. G. Palmer	111
	Summary of locus-to-locus linkage data in soybean. C. C. Yee and R. G. Palmer	115

	Summary of trisomic linkage data in soybean. R. G. Palmer 127
	A method of isolating poly(A)-containing RNS from soybean suspension culture cells for cDNA synthesis and cloning. J. Brown, R. Treick and K. Wilson
	Electrophoretic classification of selected <i>G. max</i> plant introductions and named cultivars in the late maturity group.  M. B. Gorman, Y. T. Kiang and Y. C. Chiang
	Linkage of electrophoretic loci. M. B. Gorman, Y. T. Kiang and Y. C. Chiang
	Inheritance of a second leucine amino-peptidase locus and tests of its linkage with other loci. Y. T. Kiang, Y. C. Chiang and M. B. Gorman
	Mechanical separation of seed from male-sterile and fertile plants by seed size. T. E. Carter, Jr., J. W. Burton and E. B. Huie, Jr
	Resistance to anthracnose. G. R. Bowers, Jr 150
	Soybean floral ecology and insect pollination. E. H. Erickson 152
	U.S.S.R.
	Some characteristics of lectins in soybean seeds. E. L. Golinskaya, I. V. Chernaja, V. F. Marjushkin and V. I. Sichkar . 163
	Vietnam
	Contributory applied research in growing soybean in the Mekong Delta - Socialist Republic of Vietnam. C. H. Tin
	Zambia
	Summary of national soybean variety test. J. M. Joshi, F. Javaheri and S. Nkumbula
VI.	INDEX OF AUTHORS
VII.	RECENT SOYBEAN GENETICS AND BREEDING PUBLICATIONS
VIII.	MAILING LIST

#### I. FOREWORD

In Volume 11, the biggest yet, we present several special items. On page 115 will be found a locus-to-locus linkage summary, prepared from data gathered from more than 20 publications, including many authors. Prof. Yee Chien-Chang, a visiting scholar in our laboratory last year, prepared this summary. Another summary of linkage data, presenting data of linkage among 29 mutants and the three known primary trisomics of soybean, is presented on page 127. Rhizobiologists and physiologists may be specially interested in a selection from the "World Catalogue of Rhizobium Collections" printed in this issue. We presented only a part of a large list of collections of strains of rhizobia in culture throughout the world. The catalogue is edited by V. B. O. Skerman, published in 1983.

Over the years, we have attempted to present our mailing list in the most usable manner. Even-numbered volumes list subscribers alphabetically by name; odd-numbered volumes organized the list by country and state. This year, we have encountered one of the limitations of computerized mailing systems: subscribers within the United States are listed by zip code; those outside the U.S. are presented alphabetically by individual. We hope to find time before the next issue to reorganize our data in the computer so that the most convenient presentation is more practical.

Volunteers who have made this newsletter possible include grad students and technicians Long-Fang O. Chen, Lou Forrai, Bob Graybosch, Jeff Griffin, Jeff Gwyn, Peg Hatfield, Holly Heer, Huang Jin Tai, Mary McFerson, Doug Schillinger, Randy Shoemaker, Diane Stevermer, and Susan Yost. Their loyal labor is much appreciated.

Reid G. Palmer, editor

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#### II. ANNOUNCEMENT

#### WORLD SOYBEAN RESEARCH CONFERENCE

Ames, Iowa, USA, will be the site of the World Soybean Research Conference-III, August 12-17, 1984.

Iowa State University is pleased to invite all soybean scientists to attend World Soybean Research Conference-III. The conference will provide an opportunity to discuss recent advances in research on soybean production, marketing, and utilization. Scientists in all disciplines of soybean research from throughout the world are invited to attend.

Information about the conference may be obtained by writing

Dr. Walter R. Fehr
Department of Agronomy
Iowa State University
Ames, Iowa, 50011 USA



1984

#### III. SOYBEAN GENETICS COMMITTEE REPORT

#### Minutes of the Meeting

The Soybean Genetics Committee met Monday, 13 Feb. 1984 at the Airport Hilton, St. Louis, MO. This meeting was held in conjunction with the annual Soybean Breeders Workshop.

Committee members in attendance were R. L. Bernard, H. R. Boerma, T. C. Kilen, J. H. Orf, R. G. Palmer and J. R. Wilcox. Also present was E. T. Gritton. Drs. W. D. Beversdorf and J. R. Wilcox have been elected to new three-year terms on the committee, replacing Drs. Buzzell and Hadley whose terms expired at the close of the meeting. Present committee members and the expiration of their terms are as follows.

- R. L. Bernard, Ex Officio (Curator of soybean genetics collection) Department of Agronomy University of Illinois 1102 South Goodwin Urbana, IL 61801
- W. D. Beversdorf (1987) Crop Science Department University of Guelph Guelph, Ontario Canada N1G 2W1
- H. R. Boerma (1986) Department of Agronomy University of Georgia Athens, GA 31794
- T. E. Devine (1986) Rm. 218, Bldg. 001 BARC-West Beltsville, MD 20705

- T. C. Kilen (1985) Soybean Production Research P.O. Box 196 Stoneville, MS 38776
- J. H. Orf, Chrm. (1985) Dept. of Agronomy and Plant Genetics University of Minnesota St. Paul, MN 55108
- R. G. Palmer, Ex Officio (Ed. of Soybean Genetics Newsletter) Department of Agronomy Iowa State University Ames, IA 50011
- J. R. Wilcox (1987)
  Department of Agronomy
  Purdue University
  West Lafayette, IN 47907

Dr. Orf was re-elected chairman of the committee for the coming year, so manuscripts concerning qualitative genetic interpretation and gene symbols should be sent to him for review.

At the request of the authors of the chapter on qualitative genetics and cytogenetics for the revised *Soybean Monograph*, a survey was taken of current and former members of the soybean genetics committee concerning the question of allowing gene symbol notation to be written in a computer-compatible format (all on a single line with no superscripts or subscripts). The result of the survey was 13 in favor of allowing this possibility, 2 against. This change will appear in the rules for genetic symbols.

The number of manuscripts received for review by the committee increased to 18 from 13 the previous year. Persons who are not members of the committee may be asked to review manuscripts when their area of expertise is needed and to spread the workload.

It was moved by Bernard, seconded by Boerma, to include a group of international scientists as international members of the Soybean Genetics Committee in order to foster better communication between U.S. and Canadian scientists and other scientists working on qualitative genetics. The motion was passed. A statement on the duties of international members and proposed international members will be made by the Soybean Genetics Committee.

It was suggested that anyone publishing articles that include the assigning of gene symbols send reprints of the articles to Dr. Palmer and/or Dr. Bernard.

A lengthy discussion was held concerning changes in the rules for genetic symbols. Specific changes concerning isozymes were debated. A revised list of rules that includes the changes discussed by the committee will be published in the Soybean Genetics Newsletter in 1985.

An updated list of gene symbols and T-lines will be published in the Soybean Genetics Newsletter. A note also will appear regarding the use of phytophthora gene symbols.

The committee also discussed the review of manuscripts and the assignment of gene symbols. The final choice of a symbol is up to the researcher involved provided the symbol has not been assigned previously. However, the committee hopes the rules for genetic symbols as published in the Soybean Genetics Newsletter will be followed.

Submitted by:

James H. Orf, Chairman Soybean Genetics Committee

#### A) Organization of the Committee:

- 1) The Committee will be composed of six elected members, the editor of the Soybean Genetics Newsletter, and the curator of the soybean genetics collection.
- 2) The term of the elected members will be three years. After a member has been off for one year, he (she) can be reelected. The Committee will elect two new members each year; a simple majority is needed for election. The members will be elected prior to February 1 of each year, by a mail ballot conducted by the chairman.
- 3) At the annual meeting of the Committee (usually in February in conjunction with the Soybean Breeding and Genetics Workshop), the two new members and the two retiring members of the Committee are eligible to attend and vote.
- 4) The chairman will be elected at the annual Committee meeting and serve through the next annual meeting, and may be reelected.

- B) The duties of this Committee include the following:
  - 1) Maintain Genetic Collection.

The Genetic Collection is divided into four categories:

- a) Type Collection includes all published genes of soybeans, preferably in the original strains (excluding U.S. and Canadian name varieties, which are maintained in a separate collection) plus certain mutants or strains that appear to the Committee to have potential genetic interest.
- b) Isoline Collection includes adapted varieties Clark, Harosoy and Lee, into which have been backcrossed single genes or combinations of genes. Also included are certain genes or combinations with Chippewa, Wayne and Williams.
- c) Linkage Collection includes linkage combinations and the various genetic recombinations.
- d) Cytological Collection includes translocations, inversions, deficiencies, trisomics, tetraploids, etc.

Collections a, b, and c are maintained at Urbana, Illinois, with R. L. Bernard as curator. Collection d is maintained at Ames, Iowa, with R. G. Palmer as curator.

2) Manuscript review and genetic symbol approval.

The Soybean Genetics Committee requests that researchers submit all manuscripts concerning qualitative genetic interpretation and symbols to the Committee Chairman. This review by the Genetics Committee will serve to insure orderly identification and use of genetic nomenclature and to avoid conflict of symbols. This will also allow assignment of type collection designations (T-numbers) prior to publication, so that these T-numbers may be used in the journal article to identify parental lines.

3) Soybean Genetics Newsletter notes.

All notes for the Newsletter should be sent to the SGN editor, R. G. Palmer, who will ask the Soybean Genetics Committee to review those articles concerning qualitative genetic interpretation and symbols. Genetic symbols reported in the Newsletter will have the same status as those published in scientific journals.

- C) The Committee will take the responsibility for publishing every five years, starting in 1983, in the SGN a list of all gene symbols, linkage groups, translocations, and trisomics in soybeans. Researchers who have references on the gene symbols and linkage groups are urged to send them to R. L. Bernard. Researchers who have references on translocations and trisomics are urged to send them to R. G. Palmer.
- D) The function of the Committee was officially expanded to include genetics research in the entire *Glycine* genus rather than restricting its responsibilities to *Glycine max*.

E) Researchers submitting manuscripts on new gene symbols are urged to furnish R. L. Bernard with seeds of the line carrying the reported gene. From 50 seeds to 300 gms of seed of each line are needed to maintain the genetic type collection. When these seeds are received, the genetic type number can be assigned and can then be reported by the author in a manuscript.

#### Rules for Genetic Symbols

#### I) Gene Symbols

- a) A gene symbol shall consist of a base of one to three letters, to which may be appended subscripts and/or superscripts as described below.
- b) Genes that are allelic shall be symbolized with the same base letter(s) so that each gene locus will be designated by a characteristic symbol base.
- c) The first pair of genes reported for a gene locus shall be differentiated by capitalizing the first letter of the symbol for the dominant or partially dominant allele. (Example: Ab, ab. Ab is allelic and dominant to ab.) If genes are equivalent, codominant, or if dominance is not consistent, the capitalized symbol may be assigned at the author's discretion.
- d) When more than two alleles exist for a locus, the additional alleles or those symbolized subsequently to the pair first published shall be differentiated by adding one or two uncapitalized letters as a superscript to the base. (Example: R,  $r^{m}$ , r.) This shall be the only use of superscripts. The base for the additional alleles is capitalized only when the gene is dominant or equivalent to the allele originally designated with a capitalized symbol. The superscript may be an abbreviation of a descriptive term. When allelism is discovered for a gene previously assigned a symbol, the previous symbol may be used as the superscript.
- e) Gene pairs with the same or similar effects (including duplicate, complementary or polymeric genes) should be designated with the same letter base differentiated by numerical subscripts, assigning 1, 2, 3, 4, etc., consecutively in the order of publication. (Example: The y series for chlorophyll deficiency.) This shall be the only use of subscripts. Letter subscripts should not be used. The subscript l is automatically a part of the first reported gene symbol for each base but may be omitted until the second symbol is assigned.
- f) Base letters may be chosen so as to indicate apparent relationships among traits by using common initial letters for all loci in a related group of traits. Examples are P for pubescence type, R for disease reaction (plus two initials of the pathogen to complete the base), and L for leaf shape.
- g) The distinction between traits that are to be symbolized with identical, similar, or with unrelated base letters is necessarily not clear cut. The decision for intermediate cases is at the discretion of the author but should be in accordance with previous practices for the particular type of trait. The following sections concern supplementary

- symbols that may be used whenever desired as aids to presentation of genetic formulas.
- h) A dash may be used in place of a gene symbol to represent <u>any</u> allele at the indicated locus. The locus represented should be apparent from its position in the formula. (Example: A represents both AA and Aa.)
- i) A question mark may be used in place of a symbol when the gene is unknown or doubtful, or it may be used as a superscript to the base symbol for the same purpose. (Example: a? indicates that the latter is an unknown allele at the A locus.)
- j) Plus symbols may be used in place of the assigned gene symbols of a designated standard homozygous strain when this will facilitate presenting genetic formulas. The standard strain may be any strain selected by the worker, as long as the strain being used and its genetic formula are made explicit.

#### II) Linkage and Chromosome Symbols

- a) Linkage groups and the corresponding chromosomes shall be designated with Arabic numerals. Linkage shall be indicated in a genetic formula by preceding the linked genes with the linkage group number and listing the gene symbols in the order that they occur on the chromosome.
- b) Permanent symbols for chromosomal aberrations shall include a symbol denoting the type of aberration plus the chromosome number(s) involved. Specific aberrations involving the same chromosome(s) shall be differentiated by a letter as follows: The symbol Tran shall denote translocations. Tran 1-2a would represent the first case of reciprocal translocations between chromosomes 1 and 2, Tran 1-2b the second, etc. The symbol Def shall denote deficiencies, Inv inversions, and Tri primary trisomics. The first published deficiency in chromosome 1 shall be symbolized as Def 1a, the second as Def 1b, etc. The first published inversion in chromosome 1 shall be denoted as Inv 1a, etc. The first published primary trisomic shall be designated with the Arabic numeral that corresponds to its respective linkage group number.
- c) Temporary symbols for chromosomal aberrations are necessary, as it may be many years before they are located on their respective chromosomes. Tran 1 would represent the first case of a published reciprocal translocation; Tran 2, the second case, etc. The first published deficiency shall be symbolized as Def A, the second as Def B, etc. The first published inversion shall be symbolized as Inv A, and second as Inv B, etc. The first published primary trisomic shall be designated as Tri A, the second as Tri B, etc. When appropriate genetic and/or cytological evidence is available, the temporary symbols should be replaced with permanent symbols, with the approval of the Soybean Genetics Committee.

#### III) Cytoplasmic Factor Symbols

a) Cytoplasmic factors shall be designated with one or more letters pre-fixed by cyt-. (Example: cyt-G indicates the cytoplasmic factor for maternal green cotyledons, cyt-Y indicates that for maternal yellow cotyledons.)

#### IV) Priority and Validity of Symbols

- a) A symbol shall be considered valid only when published in a recognized scientific journal, or when reported in the Soybean Genetics Newsletter, with conclusions adequately supported by data which establish the existence of the entity being symbolized. Publication should include an adequate description of the phenotype in biological terminology, including quantitative measurements wherever pertinent.
- b) In cases where different symbols have been assigned to the same factor, the symbol first published should be the accepted symbol, unless the original interpretation is shown to be incorrect, the symbol is not in accordance with these rules, or additional evidence shows that a change is necessary.

#### V) Rule Changes

a) These rules may be revised or amended by a majority vote of the Soybean Genetics Committee

#### IV. SPECIAL REPORTS

#### Rhizobium japonicum Strains Maintained in Culture

#### Collections throughout the World //

[This listing of *Rhizobium japonicum* collections was obtained from the 'World Catalogue of *Rhizobium* Collections' edited by V.B.D. Skerman\*(1983). While not a complete list of strains of *rhizobia* held in culture collections throughout the world, it is useful to rhizobiologists and others. *Rhizobium* species other than *japonicum* may be effective on *Glycine* species and are listed in the catalogue but are not included in this table.]

		Location	Head of the Collection	Curator of Collection	No. of Strains
	1.	Instituto Agrotecnico Facultad de Ciencias Agrarias Universidad Nacional del Nordeste Avenida Las Heras 727 Resistencia ARGENTINA	A. M. Castany	A. M. Castany	7
	2.	Division of Plant Industry C.S.I.R.O. P.O. Box 1600 Canberra City, A.C.T. 2601 AUSTRALIA	F. J. Bergersen	J. Brockwell	2
	3.	Australian Inoculant Research and Control Service Department of Agriculture P.O. Box 720 Gosford, N.S.W. 2250 AUSTRALIA	R. J. Roughley	G. Bullard	4
4	4.	C.S.I.R.O. Division of Tropical Agronomy Cunningham Laboratory Mill Road St. Lucia 4067 Queensland AUSTRALIA	R. A. Date	R. B. Panitz	4
5	5.	Faculte des Sciences Agronomiques de l'Etat Chaire de Microbiologie B-5800 Gembloux BELGIUM	C. Bonnier	D. Cornet	2
6	5.	Empresa Brasileira de Pesquisa Agropecuaria Servico Nacional de Levantamento e Conservacao de Solos Programa Fixacao Biologica de Nitrogenio kn47, 23460 Seropedica Rio de Janeiro BRAZIL	J. Bobereiner	A. A. Franco	9

	Location	Head of the Collection	Curator of Collection	No. of Strains
7.	Microbiological Resources Center (MIRCEN) Instituto de Pesquisas Agronomicas Secretaria da Agricultura Rua Goncalves Dias, 570 90.000 Porto Alegre, RS BRAZIL	J. R. Jardim Freire	E. Brose	4
8.	National Agro-Industrial Union N. Poushkarov Institute of Soil Science Shosse Bankja Str. No. 5 Sofia BULGARIA	L. Glogov	L. Raicheva	29
9.	Laboratory of Soil Microbiology Institute of Soils and Fertilization Chinese Academy of Agricultural Sciences Beijing (81) PEOPLE'S REPUBLIC OF CHINA	T. S. Hu	K. Z. Nín	4
10.	Laboratory of Soils and Fertilization Institute of Oil Crops Chinese Academy of Agricultural Sciences Wuhan PEOPLE'S REPUBLIC OF CHINA	P. C. Chou		1
	Department of Soil Microbiology Research Institute of Crop Production 161 06 Praha 6-Ruzyne CZECHOSLOVAKIA	H. Mareckova	M. Slepickova	20
11b.	Department of Bacteriology State Laboratory for Soil and Crop Research Lottenborgvej 24 DK-2800 Lyngby DENMARK	T. V. Nissen	M. Schroder	3
12.	Laboratoire de Microbiologie del Sols 17, Rue Sully, B.V. 1540 21034 - Dijon Cedex FRANCE	N. Amarger	M. Bours	4

	Location	Head of the Collection	Curator of Collection	No. of Strains
13.	Sektion Biowissenschaften der Karl-Marx-Universitat 7010 Leipzig, Talstrasse 33 GERMAN DEMOCRATIC REPUBLIC	G. Schuster	G. Menzel	1
14.	National Institute for Agricultural Quality Testing Budapest, Keleti Karoly 24 HUNGARY	E. Bakondi-Zamory		2
15.	Research Institute of Soil Science and Agricultural Chemistry Hungarian Academy of Sciences 1022 Budapest Hermann Otto es-15 HUNGARY	E. Manninger		1
16.	PHYLAXIA Veterinary Biologicals and Feedstuffs 1486 Budapest, Szallas ul 5 HUNGARY	T. Soos		2
17.	Department of Soil Science and Agricultural Chemistry J.N.A.U., P.B. No. 80 Krishinangar 482004 Jabalpur INDIA	J. N. Dube	U. K. Vashya E. Joseph	3
18.	Biochemistry Division National Chemical Laboratory Pune 411 008 INDIA	S. R. Modak		2
19.	Indian Agricultural Research Institute Division of Microbiology New Delhi 110012 INDIA	N. S. S. Rao	S. K. Kavimandam	15
20.	Rhizobium Collection Department of Soil Science Universiti Pertanian Malaysia Serdang Selangor MALAYSIA	Z. H. Shamsuddin	Z. H. Shamsuddin	39
21.	International Institute of Tropical Agriculture Oyo Road, PMB 5320 Ibadan NIGERIA	A. Ayanaba	K. Mulongoy	43

	Location	Head of the Collection	Curator of Collection	No. of Strains
22.	Soil Biology Section Soil Research Division Alabang Central Soil Research Station Alabang, Muntinlupa, Rizal PHILIPPINES	F. M. Lapid L. C. Francisco	N. Gonzales	1
23.	Department of Microbiology Institute of Soil Science and Plant Cultivation 24-100 Pulawy POLAND	W. Maliszewska	T. Wrobel	3
24.	Research Institute for Cereals and Technical Plants Laboratory of Soil Biology Boulevard Marasti 61, Bucharest ROUMANIA	N. Balan	E. Galbenu	10
25.	Plant Protection Research Institute Private Bag X134 Pretoria 0001 SOUTH AFRICA	B. W. Strijdom	C. J. Otto	7
26.	Thailand Institute of Scientific and Technological Research 196 Phahonyothin Road Bangken, Bangkok 9 THAILAND	P. Atthasampunna	S. Chomchalow	21
27.	Rothamsted Collection of Rhizobium Soil Microbiology Department Rothamsted Experimental Station Harpenden Hertfordshire UNITED KINGDOM	J. E. Beringer	M. Dye	11
28.	USDA ARS Rhizobium Collection Beltsville, Maryland 20705 USA	D. F. Weber H. H. Keyser		8
29.	University of Hawaii College of Tropical Agriculture and Human Resources Department of Agronomy and Soil Science NIFTAL Project P.O. Box O Paia Hawaii 96779 USA	A. S. Whitney	P. Somasegaran	11

	Location	Head of the Collection	Curator of Collection	No. of Strains
30.	Institute of Microbiology Armenian SSR Academy of Sciences Abovian City 37850 Armenian SSR USSR	A. D. Nalbandian	J. S. Melkonian	2
31.	Institute of Experimental Biology Estonian SSR Academy of Sciences 203051 Harku sj., Harju rajoon Estonian SSR USSR	E. Parsim	E. Parsim E. Lokk	1
32.	All-Union Scientific Research Institute of Agricultural Microbiology Sh. Podbelskogo 3, 188620 Leningrad, Pushking USSR	O. A. Berestetski	A. T. Novíkova	37
33.	Laboratory of Microbiology Ukrainian Scientific Research Institute of Agriculture 255205 Chabany, Kievskaya oblast Ukrainian SSR USSR	I. N. Romeiko	E. K. Dubovenko S. M. Malinskaja L. N. Chechelniska	2 ja
34.	University of Can Tho Tropical Biological Nitrogen Research Center Hau Giang VIETNAM	T. P. Duong	L. T. K. Nhan	1
35.	Laboratory of Microbiology Faculty of Agriculture 71.000 Sarajevo YUGOSLAVIA	V. J. Radulovic	M. Poplasen	4

<sup>\*</sup> Address of editor: V.B.D.Skerman, World Data Center for Microorganisms, Department of Microbiology, University of Queensland, St. Lucia, Queensland, 4067, Australia.

#### SOYBEAN GERMPLASM ADVISORY COMMITTEE

The Soybean Germplasm Advisory Committee was established to advise the soybean germplasm curators and others involved in soybean germplasm policy and administration. In an attempt to be responsive to the needs of the research community, the committee has been expanded to fourteen members. Five members are ex officio: The curators of the southern and northern portions of the soybean germplasm collection, the two USDA research geneticists working with the collection, and a representative of the USDA National Program Staff. The remaining nine members are elected for 3-year terms and represent the following geographic and/or research areas:

- 1. Private breeder, north
- 2. Private breeder, south
- 3. Public breeder, north
- 4. Public breeder, south
- 5. Pathologist or nematologist
- 6. Pathologist or nematologist
- 7. Entomologist
- 8. Physiologist or biochemist
- 9. Cytogeneticist or molecular geneticist

The following people are currently members of the committee: E. E. Hartwig, R. L. Bernard, T. C. Kilen, R. L. Nelson, P. A. Miller, C. W. Jennings, C. Williams, W. R. Fehr, K. Hinson, S. M. Lim, R. A. Kinloch, M. J. Sullivan, R. F. Wilson, and R. G. Palmer. R. L. Nelson is the current chairperson and T. C. Kilen is the vice-chairperson. Any comments regarding soybean germplasm in the U.S. would be welcomed by any member of the committee.

The committee met on February 13, at St. Louis, Missouri. Four major items were on the agenda.

A report was given on the status of the germplasm evaluation and enhancement proposals submitted by the committee to the USDA in March 1983. It was intended that these proposals would compete with proposals from other commodities for funding in FY 85. However, it now seems highly unlikely that money will be available for such a project. The committee is concerned about the lack of support for germplasm research and voted to express this concern to appropriate individuals.

Reports were given of the status of both the northern and southern portions of the USDA Soybean Germplasm Collection. A detailed germplasm report is given elsewhere in this volume and will not be covered in this report.

The committee considered a request from the USDA Plant Exploration and Taxonomy Laboratory to assess the needs for soybean germplasm exploration. The exploration priorities recommended by the International Board of Plant Genetic Resources (IBPGR) Working Group on the Genetic Resources of Glycine species were reviewed. Through funding from IBPGR, a project is currently underway to create a directory of soybean germplasm collections worldwide. The final recommendations from the committee will wait until this directory has been completed.

Soybean germplasm exchange with the People's Republic of China was discussed. Frustration was expressed at the failure to achieve major soybean germplasm exchanges, although we continue to receive some new accessions each year. The committee will be working to increase germplasm exchange between the two countries.

--R. L. Nelson

U.S. SOYBEAN GERMPLASM COLLECTION REPORT

Listed below are the new additions to the Germplasm Collection grown at Urbana, Illinois, in 1983:

Country of Origin	Second year	New
China	155	2
Sweden	2	
USSR		10
S. Korea		3

The second year additions bring the Urbana Collection to the following totals:

Maturity Group	0ld varieties	FC strains	PI strains	<u>Total</u>
000	3	1	89	93
00	5	4	327	336
0	7	6	819	832
I	23	3	1108	1134
II	26	6	1177	1209
III	38	13	1074	1125
IV	_38_	18	2302	2358
Total	140	51	6896	7087

The total number of accessions at Urbana by country of origin is as follows:

Origin	1980 & earlier	Entered since 1980	Total	Percentage
China	1072	197	1269	17.9
Japan	1068	4	1072	15.1
Korea	1827	219	2046	28.9
Other Asian	17	-	17	.2
USSR	1803	-	1803	25.5
Europe	759	2	761	10.7
Other	119		119	1.7
	6665	422	7087	100.0

A new checklist by entry and maturity group was recently prepared and is available from the curator.

A Soybean Germplasm Collection Inventory covering all maturity groups and all accessions received through 1980 has been prepared and has been submitted for review prior to publication. This should be especially convenient for those using PI strains in their research and wishing to identify the country of origin and variety name.

A survey is being made of the status of current soybean germplasm collections worldwide. Over 180 individuals in 80 countries were sent a question-naire requesting information on the location, type, number of accessions, storage facilities, and seed availability of their germplasm collection. Responses are now being compiled and a complete directory will be available in late 1984.

The second year of agronomic evaluations was completed for approximately 600 accessions in maturity group 0 or earlier and over 800 accessions in groups I, III, and IV. A test of over 450 group II accessions was lost due to adverse weather conditions and will be repeated in 1984. Since 1980, approximately 4,500 accessions in maturity groups IV and earlier have been evaluated with the help of Dr. J. W. Lambert, University of Minnesota, and Dr. J. H. Orf, University of Kentucky and University of Minnesota. Publications containing this information are currently being prepared.

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1)  $\frac{1}{2}$  Wild perennial Glycine species as genetic resources for soybean improvement.

The soybean genus *Glycine* is currently divided into two subgenera, *Glycine* and *Soja* (Hymowitz and Newell, 1981). The subgenus *Soja* includes soybean (*G. max*) along with its annual wild progenitor (*G. soja*), with which soybean can be readily crossed. The other subgenus, *Glycine*, contains at least seven perennial wild species, all of which occur in Australia. The five diploid species, *Glycine latifolia*, *G. latrobeana*, *G. canescens*, *G. clandestina* and *G. falcata* (all 2n = 40), occur only in Australia, whereas *G. tabacina* (2n = 40, 80) and *G. tomentella* (2n = 38, 40, 78, 80) are more widespread. All cytological races of these two species occur in Australia, diploid *G. tomentella* (2n = 40) occur in Papua New Guinea, and tetraploid strains of both species are found in South Pacific Islands, Philippines, Taiwan, and Southern China.

Because of the continent-wide distribution of the subgenus, and their presence in a wide array of habitats, these species possess many characteristics which could be economically important if transferred to soybean. These include drought and cold tolerance, apparent day-length insensitivity and disease resistance, especially resistance to soybean leaf rust (*Phakopsora pachyrhizi*). This pathogen causes serious yield losses in Asia and Australia, yet current sources of resistance within *G. max* are very limited. In 1975, the Division of Plant Industry, CSIRO, began a research program to collect, study, and exploit the potential resources in the wild perennial species. The recent results of some aspects of this program are summarized below.

## 1) Interspecific hybridization between soybean and its wild perennial relatives

A key step to using the genetic resources of the wild species is to obtain crosses between the perennials and the cultivated annuals. Until recently, such crosses had invariably aborted, usually because the hybrid endosperm failed to develop. Now, however, hybrid plants have been produced by in vitro culture of the hybrid embryos obtained by pollinating complex wild amphiploids with soybean pollen (Broué et al., 1982), or by using tetraploid G. tomentella pollen on soybean (Newell and Hymowitz, 1982). Such plants are sterile with highly irregular meiosis. They are perennial and readily propagated vegetatively.

The hybrids obtained in Canberra were produced from crosses between cultivars 'Lincoln' or 'Hark' with the synthetic amphiploid (2n = 78) of two wild species [Glycine tomentella  $(2n = 38) \times G$ . canescens (2n = 40)]. The plants had 59 chromosomes; one genome from each of the three contributing species. They expressed resistance to soybean leaf rust inherited from the G. canescens parent.

The chromosome number of the hybrid has been doubled using colchicine. However, this step did not restore significant pollen fertility, although occasional fertile grains were seen. No selfed seed has been obtained. Examination of meiosis in pollen mother cells in the doubled

hybrid revealed a high frequency of multivalent associations. Indeed, the pollen fertility of the amphiploid wild parent was incomplete (70%) and the selfed seed set was erratic. Meiosis in the wild amphiploid also showed multivalent associations. Extensive efforts at backcrossing the doubled hybrid using wild or soybean pollen have so far failed to produce pods. The pollen fertility of the hybrids was then tested at three different temperature regimes and two daylengths, but with no significant improvement after six weeks.

The fertility problems encountered with this first hybrid have given added impetus to the program of crossing soybean with other wild accessions. Hybrid plants in culture have been obtained from at least four other diverse wild species, and these are being established in the glasshouse.

#### 2) Systematic relationships within the subgenus Glycine

Crossing relationships among and within the perennial species of Glycine have been studied to understand the evolutionary history of the genus, and to develop pathways for transferring useful characters from any of the more distant wild species to soybean itself (Broué et al., 1979). Putievsky and Broué (1979) reported the meiosis in five interspecies crosses, and Newell and Hymowitz (1983) confirmed and extended their results including a new interspecific combination. We are now to report on twelve new hybrid combinations among eight perennial species (Grant et al., 1984). These data, together with those of the two previous studies, showed the close genomic relationships among the diploid species G. canescens, G. clandestina, G. latrobeana, and the euploid tetraploid G. tomentella. Distant from this group of species is a second branch which includes the diploids G. latifolia, and an undescribed species G. "reducta". The diploid G. tomentella (2n = 38, 40) appeared to be intermediate between these two branches. Interspecies hybrids using either the diploid species G. falcata, or the tetraploid G. tabacina have so far been achieved only with members of the G. canescens-related group, from which both species are very distant. Their close relatives are, therefore, presently unknown.

# 3) Host-pathogen relationships between soybean leaf rust and *Glycine* species

Surveys of accessions of perennial *Glycine* species for reaction to an isolate of *Phakopsora pachyrhizi* (Burdon and Marshall, 1981) have demonstrated that the wild species displayed considerable diversity for resistance to this pathogen. More recently, eight new isolates were obtained from the soybean fields from coastal New South Wales and Queensland. They were tested for pathogenicity on a wide range of wild *Glycine* accessions (Burdon and Speer, 1984). The results revealed the existence of six pathotypes of the rust fungus, and, simultaneously, allowed the definition of a differential set of *Glycine* accessions which can be used to classify any new isolate according to pathotype. Such a set of lines was until now entirely lacking for this pathogen because of the very low levels of resistance generally found within *G. max*. The newly designated lines will now allow a study of the genetic basis of resistance and, more generally, the coevolution of host and pathogen in natural communities.

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150

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1) Effects of sowing date and decapitation on green soybean/

Green soybean as fresh vegetable is gaining popularity in many of the soybean-producing countries of the world. Soybean as vegetable can be grown easily during rainy season because final product is harvested as immature green seed (Shanmugasundaram et al., 1982). Decapitating at 4-5 trifoliolate leaves has been found to increase the yield by 14 to 22% (Tin, 1982). It was aimed in the present experiment to ascertain the date of sowing during the rainy season of Assam and the stage of decapitation of green soybean.

An experiment with treatments of two dates of sowing (July 20 and August 1) and two stages of decapitation (6 leaves and 7 leaves) and without decapitation was undertaken in the Assam Agricultural University, Jorhat, India, during 1983. The ninth generation of the cross material SC 7601 (yellow-seeded 'Ankur' X black-seeded 'Himso 330') was used. It was laid out in six randomized blocks. Each plot (4m x 3m) received manure and fertilizers at the rate of 5q poultry manure, 20 kg N as urea, 60 kg  $P_2O_5$  as single superphosphate, 40 kg  $K_2O$  as muriate of potash and 2 t lime as  $CaCO_3$  per hectare. Seeds treated with rhizobium culture (5 g/kg seed) were sown at 60 kg/ha in lines 45 cm apart.

Sowing seeds either on July 20 or August 1 did not significantly influence the growth and yield attributing characters (Table 1). Nevertheless, the yields of green beans and grains per plant, as well as green beans/ $m^2$  were significantly more with July 20 sowing.

The number of branches and pods per plant were significantly more when decapitated at 6 or 7 leaves stages compared to those of normal plants (Table 1). Seeds were heavier in plants decapitated at 7-leaf stage, other treatments being at par. Decapitation at 7-leaf stage significantly increased the yields of green beans and grains per plant as well as green beans/ $m^2$  over those under the plants decapitated at 6-leaf stage and in these respects both the treatments proved superior to normal plants.

Decapitation at 7-leaf stage significantly increased the yield of green beans and green grains/plant as well as green grain/ $m^2$  for both July 20 and August 1 sowing, compared with those of normal plants and also recorded better values over those of the treatment decapitation at 6-leaf stage (Table 2). In general, July 20 planting with decapitation at 7-leaf stage appeared to be better in regard to yields of green beans and grains.

Cooking quality of green grains was tested. Seeds were boiled in 0.05% solution of  $Na(CO_3)_2$  and washed with cold water, then again boiled for 10 minutes in pressure cooker. The softness of the grains harvested 35 days, 45 days and 55 days after flowering was rated as very soft, soft and slightly hard, respectively, and these were much palatable.

Effects of sowing dates and decapitation on growth, yield attributes and yield soybean Table 1.

							Yield	Yield (g) of		
	Plant	No. of	No. of	100-see	100-seed weight at 75 (g) at	Green bean/	Green grain/	Green bean/	Grain/ m <sup>2</sup> at	Days to
Treatments	height (cm)	branch/ plant	pod/ plant	days (green)	maturity (dry)	plant	plant (at 7	int m <sup>2</sup> (at 75 days)	maturity	maturity
Date of planting (P)	Ing									
July 20	80.84	8,33	91.56	31.58	16.77	163.45	93.43	1630.00	335.74	115
August 1	82.21	8.82	89.33	31.83	16.87	156.33	89.17	1556.67	333.73	110
SEd	1.09	0.39	1.48	0.27	0.26	2.19	1.40	16.24	3.45	ı
CD(5%)	N.S.a	N.S.	N.S.	N.S.	N.S.	4.51	2.90	33.47	N.S.	ı
Decapitation (D)	( <u>a</u> )									
Normal plant	80.15	7.50	83.09	31.16	16.53	147.52	84.08	1470.00	330.00	115
6-leaf stage	82.75	8.58	92.43	31.59	16.84	161,31	92.22	1610.00	336.80	117
7-leaf stage	81.68	9.65	95.77	32.37	17.10	170.84	97.59	1700.00	342.00	119
SEd	1.33	0.48	1.81	0.33	0.32	2.68	1.72	19.90	4.23	ı
CD(5%)	N.S.	0.99	3.73	69.0	N.S.	5.53	3.55	40.99	N.S.	ı

 $^{a}N.S. = nonsignificant.$ 

Interaction effects of dates of planting and decapitation on soybean Table 2.

Mature grain/m²	328.00	334.40	344.00	332.00	339.20	340.00	4.23	8.73
Green grain/ m <sup>2</sup>	1430.00	1680.00	1780.00	1510.00	1540.00	1620.00	28.14	57.98
Yield (g) of Green grain/ plant (at 75 days)	81,86	96.21	102.21	86.30	88.23	92.98	2.44	5.03
Green beans/	143.25	168.22	178.88	151.78	154.40	162.80	3.79	7.82
100-seed weight (g) (green)	30.89	31,33	32.52	31.43	31.86	32.21	0.47	66.0
Number of pods/ plant	80.55	95.22	98.92	85.63	89.74	92.62	2.56	5.28
Number of branches/ plant	7.6	۳ « ه	8.1	7.4	<b>∞ ∞</b>	10.2	1	N.S.b
Plant height (cm)	77.80	81.50	83.20	82.50	84.00	80.10	1.88	3.87
Treatment combinations	P <sub>1</sub> D <sub>0</sub>	$P_1$ $D_1$	$^{\rm P}_1$ $^{\rm D}_2$	$^{\mathrm{P}_2}$ $^{\mathrm{D}_0}$	$P_2$ $D_1$	$^{P}_2$ $^{D}_2$	SEd	CD(5%)

 $^{a}_{2}$  = July 20 planting;  $^{p}_{2}$  = August 1 planting;  $^{0}_{0}$  = Normal plant;  $^{0}_{1}$  = Decapitated at 7-leaf stage.

 $b_{N.S.}$  = nonsignificant.

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### 2) Effect of varieties and date of sowing on the growth and yield of soybean

The recognition of highly valued soybean in India is well understood by its cultivation of 6 lakhs hectares during 1980-81 (Bhatnagar, 1980-81). Though Assam is famous as a rice growing area, the typical uplands are not properly utilized for rice because of partial to nonavailability of irrigation water. These areas are either partly used for raising rice seedlings or put to summer vegetables. Thus, land availability for the cultivation of soybean appears to be bright. For its widespread cultivation, varieties suitable to different agro-climatic zones of India are continued to be identified. Nevertheless, a good variety would express its full yielding potential when optimum agronomic practices prevail and, in this regard, date of sowing is second to none of the practices.

The present experiment under All India Coordinated Research Project on Soybean, comprising the four varieties (PK-271, PK-308, DS 73-16 and Bragg) and three dates of sowing (June 5, July 5 and August 5), was undertaken in the Assam Agricultural University, Jorhat, during Kharif season of 1982. The experiment was laid out in the slightly acidic (pH 6.5 due to continuous liming at 1 ton /ha/year) sandy loam soil by adopting randomized block design with three replications. The plots of 6m x 3.15m were fertilized with 20 kg N, 60 kg  $P_2O_5$  and 40 kg  $K_2O$  per hectare rates. Rhizobium-inoculated seeds at the rate of 70 kg/ha were sown in 6m long rows spaced out at 45 cm. After sowing, plots were mulched with straw in order to prevent the beating action of rains. At each date of sowing, a nursery adjacent to experimental field was also sown for the purpose of replacing seedlings of the main field in the event of mortality. Two weedings, at 15 and 30 days after sowing, were done; the second weeding was followed by a light earthing up. Nuvan (0.01%) was sprayed against Bihar hairy caterpillar.

Effect of varieties and dates of sowing on yield attributes and yield of soybean Table 1.

Treatments	Plant height (cm)	Number of branches/ plant	Number of pods/	Length of pod (cm)	Number of seeds/	100-seed weight (g)	Grain yield (g/ha)	Days to maturity
Varieties								
PK-271	77.00	06.4	190.81	4.36	2.43	13.21	27.47	110.44
PK-308	81.22	8.87	124.07	4.32	2,42	12.62	21.92	109.77
DS 73-16	98.44	4.48	178.58	4.18	2.37	11.78	24.57	107.00
Bragg	119.57	5.63	169.74	4.15	2.48	14.67	22.62	113.44
CD 5%	7.15	1.07	24.05	N.S.a	N.S.	0.73	3.19	3.27
Dates of sowing								
June 5	101,08	4.33	96.13	2.30	79.7	12.14	19.58	121.08
July 5	103.54	6.22	206.50	2.43	04.4	12.91	27.00	106.91
August 5	77.55	7.36	194.78	2.55	3.72	13.81	25.86	102.49
CD 5%	6.18	0.93	20.83	N.S.	0.53	0.85	2.76	2.83

a<sub>N.S.</sub> = nonsignificant.

The standard variety 'Bragg' registered significantly more plant height among the varieties tested (Table 1). Both 'PK-271' and 'PK-308', being at par, recorded significantly shorter plant height as compared with that of 'DS 73-16'. The variety PK-308 recorded significantly greatest number of branches per plant while the differences in such numbers between the varieties PK-271 and DS 73-16 and that between PK-271 and Bragg were nonsignificant. The difference in the number of pods/plant between the varieties PK-271 and DS 73-16and that between DS 73-16 and Bragg were significant, but all of them proved superior to PK-308. The other yield-attributing characters like the length of pod and the number of seeds per pod did not vary due to different varieties. The variety Bragg recorded significantly higher 100-seed weight than other varieties. Though the varieties PK-271 and PK-308 were at par but recorded significantly more 100-seed weight than that of the variety DS 73-16. fore, the variety PK-271, with more number of pods per plant and reasonably better 100-seed weight resulted in significantly highest grain yield (27.47 q/ha) as compared with other varieties. The other varieties did not produce any significant differences in yield. The variety PK-271, with about 110 days duration, out-vielded the standard variety Bragg.

Plant heights of June 5 and July 5 plantings were about equal, but the later August 5 planting resulted in significantly shorter plants as compared with earlier dates (Table 1). The number of branches, on the other hand, significantly increased with every advancement of sowing dates.

The difference of number of pods per plant due to July 5 and August 5 sowing was nonsignificant, but both of them proved superior to June 5 sowing. The length of pod was significantly depressed due to August 5 sowing as compared with earlier dates, which were at par in this regard. The number of seeds per pod due to different dates of sowing were nonsignificant. The effects of earlier two dates of sowing on the 100-seed weight were statistically similar, but the later date of August 5 sowing registered significantly more weight as compared with former dates. Therefore, July 5 and August 5 sowing with more number of pods per plant consequently did not vary in respect to yield. The June 5 sowing registered lowest yield and also lengthened the life span of the crop significantly.

The interaction effect of varieties and date of sowing did not affect the yield significantly.

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3) Effect of varieties and population densities on the growth and yield of soybean

The varieties of a crop with their differential genetical makeup exhibit wide variation in regard to both growth habits and ultimate yield. The maintenance of optimum plant population will not only provide ample scope for proper growth of a variety but will largely shape the ultimate yield, because the yield of a crop in general is a function of yield per plant and plant population per unit area. Experimental evidence is available to show that optimum plant populations per unit area for different soybean varieties are not the same (Singh et al., 1974; Narayana, 1976; Reddy and Singh, 1976; Deshmukh et al., 1977).

A field experiment, under All India Coordinated Project on Soybean, comprising six varieties and three population densities was conducted during July to October of 1982 at the Instructional cum Research Farm of the Assam Agricultural University, Jorhat. The soil of the experimental site was slightly acidic (pH 6.5) sandy loam. The experiment was laid out in a split plot design with three replications where varieties were alloted to the main plots and population densities to the sub-plots. The Rhizobium-treated seeds were sown in 6m-long rows laid out at 45 cm apart in the plots of 6m x 3.15m. The number of plants per plot accommodated were 378, 756 and 1134 so as to maintain plant population of 0.2 million, 0.4 million and 0.6 million per hectare, respectively. The plots were fertilized with 20 kg N, 60 kg  $P_2O_5$  and 40 kg  $K_2O/h$  ha rates. On the day of sowing, a strip was also sown with different varieties for the purpose of gap filling in the main field when required. After sowing, straw mulch was applied to protect the seeds from the beating action of rains.

The variety 'Bragg' had significantly higher plant height as compared with all 'PK' varieties and DS 73-16 (Table 1). The plant height of DS 73-16 was significantly higher than those of PK varieties but was at par with that of PK-271 only. The varietal differences in regard to number of branches per plant, pods per plant, and number of seeds per pod were nonsignificant. But the 100-seed weight of the varieties PK-271, PK-327, and Bragg were significantly more than those of the varieties PK-262, PK-308, and DS 73-16. The variety PK-262 had, however, significantly higher 100-seed weight compared with PK 308 and DS 73-16; the latter varieties were at par in this regard. The yield differences of the varieties PK-262 and PK-271 were nonsignificant but their yield levels were significantly more than those of the other varieties tested. The yield of the variety PK-327, though, appeared to be at par with that of PK 308 but was significantly superior to Bragg and DS 73-16. However, the yield levels of PK-308, Bragg and DS 73-16 were at par.

The variable plant population significantly affected none of the characters but the number of pods per plant and yield. The number of pods per plant was significantly more with 0.2 million population as compared to higher population rates, viz., 0.4 million and 0.6 million per hectare which in turn were at par. The increase of plant population from 0.2 million to 0.4 million did not bring about any significant yield difference, but further increase to 0.6 million plant population significantly increased the yield as compared to lower populations.

The interaction effect of varieties and plant population was found to be significant on number of seeds per pod, 100-seed weight and on grain yield. Here, the interaction effect on yield is discussed (Table 2). Within 0.2 million plant population, the varieties DS 73-16, Bragg and PK-271 yielded at par; the yield levels of these varieties were significantly more than those of other

Table 1. Effect of varieties and plant population on the yield attributes and yield of soybean

	Plant height (cm)	No. of branches/ plant	No. of pods/plant	No. of seeds/ pod	100-seed weight (g)	Grain yield (q/ha)
Varieties (	V)					
DS 73-16 PK-262 PK-271 PK-308 PK-327 Bragg	94.53 65.64 78.73 70.62 67.37 149.49	5.4 4.4 5.6 6.0 5.0 5.9 N.S. <sup>a</sup>	112.58 76.15 74.78 112.35 64.40 98.35 N.S.	2.3 2.3 2.4 2.4 2.2 N.S.	11.95 13.29 14.09 12.02 14.27 14.59	18.95 27.28 26.30 20.45 22.25 19.28
Plant popula	ation (P)					
0.2 m/ha <sup>b</sup> 0.4 m/ha 0.6 m/ha CD 5%	86.15 90.83 86.21 N.S.	5.5 5.5 5.2 N.S.	100.31 84.23 84.76 14.53	2.4 2.3 2.3 N.S.	13.42 13.48 13.21 N.S.	21.27 21.78 24.20 1.88

<sup>&</sup>lt;sup>a</sup>N.S. = nonsignificant.

Table 2. Interaction effect of varieties and plant population on the yield of soybean

Varieties	Plant populat	ion (P) in millior	n per hectare	
(V)	0.2	0.4	0.6	Mean
DS 73-16	22.66	17.53	16.67	18.95
PK-308	16.36	18.25	26.75	20.45
Bragg	26.55	15.96	15.33	19.28
PK-271	25.73	27.46	25.71	26.30
PK-327	18.35	22.40	26.00	22.25
PK- 262	18.01	29.06	34.76	27.28
Mean	21.27	21.78	24.20	
		CD 5%		
	V at the same leve			
	P at the same leve	$e1  ext{ of } V = 4.68$		

 $<sup>^{\</sup>rm b}$ m/ha = million per hectare.

varieties tested. With 0.4 million plant population, PK-271 and PK-262 produced statistically similar yields but were significantly superior to other varieties. At 0.6 million population level, the yields of PK-262 was significantly increased over that of any other varieties. It may further be noted that the yield levels of PK-308 and PK-327 became at par with that of PK-271 under this high population level. By increasing the population from 0.2 million to 0.4 million, the yield level significantly depressed in case of the varieties DS 73-16 and Bragg. However, PK-271 maintained the uniform yield level at any given plant population. For varieties like PK-262, PK-308, and PK-327, the yields continued to increase with the increase in plant population and so highest population of 0.6 million resulted in higher yield. From these yield trends it may be inferred that there is still scope for increasing the plant population for the varieties PK-262, PK-308, and PK-327.

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# 4) Effect of varieties and plant population on grain yield and two yield attributes of soybean,

A field experiment was conducted during rainy seasons of 1980 and 1981 to study the effect of varieties and plant population on yield of soybean at the Agronomy Farm, Assam Agricultural University, Jorhat. The seeds were sown on 30 June during both the years. The soil of the experimental plots was sandy loam with pH 5.3. Four varieties ('PK-71-21'; JS-72-375', 'JS-2' and 'Bragg') were included for study. The seeds were sown at a distance of 45 cm between rows, 3.3, 5.0 and 10.0 cm between the seeds in order to accommodate 0.6 million, 0.4 million and 0.2 million plant population, respectively. A split plot design with three replications with varieties in the main plots and plant population in the sub-plots was adopted. A basal dose of fertilizers (20 kg N/ha, 60 kg  $P_2O_5$ /ha and 40 kg  $K_2O$ /ha) was applied before sowing. Data on yield, and two yield attributes, as affected by various treatments, are presented in Table 1.

Table 1. Effect of varieties and plant population on grain yield (q/ha) and two yield attributes of soybean

	Grain yiel	d (q/ha)	100-grain	weight (g)	Number o	
Treatments	1980	1981	1980	1981	nodules/	
					1980	1981
Varieties						
PK-71-21	18.58	28.77	14.90	15.99	44.85	44.48
JS-72-375	17.51	25.39	13.45	13.46	40.60	36.70
JS-2	18.04	27.50	14.49	14.78	44.34	36.10
Bragg	20.83	31.59	15.90	15.07	50.04	41.66
CD = 0.05	2.39	3.11	2.08	1.94	4.2	4.95
Plant popul	ation					
0.2 m/ha	17.08	28.81	14.68	14.75	42.88	40.26
0.4 m/ha	19.18	26.58	14.67	14.78	43.29	39.00
0.6 m/ha	20.71	29.55	14.69	14.93	44.69	42.04
CD = 0.05	N.S. <sup>a</sup>	N.S.	N.S.	N.S.	N.S.	N.S.

<sup>&</sup>lt;sup>a</sup>N.S. = not significant.

The differences in grain yield due to varieties were significant. The grain yield of Bragg, being at par with PK-71-21, was significantly more than those of the varieties JS-72-375 and JS-2. The yield differences of the varieties PK-71-21, JS-72-375 and JS-2 were nonsignificant. The yield of Bragg was 20.85 q/ha and 31.59 q/ha during 1980 and 1981, respectively. Grain yield of Bragg and Pk-71-21 was about 16.0 and 5.7% and 20.0% and 11.7% higher during 1980 and 1981, respectively, over JS-72-375 which produced the lowest grain yield. Differences in grain yield among the varieties may be attributed principally to the differences in test weight of 100 seeds and number of root nodules per plant. The perusal of the data in Table 1 would reveal that, among all the four varieties, Bragg and PK-71-21 registered the higher 100-seed weight and greater number of nodules per plant. These two, being the varietal characters, showed wide range of differences among varieties which were reflected in differences in grain yield. Choudhury and Wamanan (1976) and Narayana (1976) also observed a similar varietal difference in soybean in respect to grain yield.

During both years, varying levels of plant populations did not affect the yield significantly. Weight of 100 seeds and number of root nodules per plant were also not affected by the variables studied. Increasing the plant population from 0.2 to 0.6 million might have increased the intrarow plant competition to such an extent that the possible effect of increased plant population on the per-hectare yield was nullified by reducing the favorable effects on other yield parameters. Several workers (Singh et al., 1974; Reddy and Singh, 1976; Pant and Joshi, 1977) have also reported nonsignificant results by increasing the plant population from 0.2 to 0.6 million on the yield of soybean.

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## 5) Performance of winter soybean under varying levels of irrigation

Possibility of growing soybean during winter season has been revealed by several workers (Sarmah, 1979; Mandloi and Tiwari, 1971). But the success of a winter crop depends mainly on available soil moisture. The average rainfall for the last ten years at the station during winter months (November to March) was 104.28 mm, which is not enough to sustain a good crop of soybean. The present investigation, therefore, was undertaken to study the influence of irrigation levels on yield performance of four soybean cultivars.

Field experiment was conducted during winter 1981-82 on medium low land rice soil of pH 5.30 having clay loam texture at the Instructional-cum-Research Farm of Assam Agricultural University, Jorhat. The treatments consisted of three levels of irrigation ( $I_1$ -pre-sowing irrigation,  $I_2$ -pre-sowing irrigation followed by one irrigation at 40 mm Cumulative Pan Evaporation (CPE),  $I_3$ -pre-sowing irrigation followed by two irrigations at 40 and 75 mm CPE) and four soybean cultivars, DS 73-16 ( $V_1$ ), JS-2 ( $V_2$ ), JS 72-375 ( $V_3$ ), and PK-271 ( $V_4$ ). The experiment was conducted in split-plot design with three replications, keeping irrigation levels in the main plot and varieties in the sub-plots. The crop received a basal application of 20 kg N, 60 kg  $P_2O_5$  and 40 kg  $K_2O/ha$  at the time of final land preparation. Soybean seed (80 kg/ha) inoculated with proper rhizobium culture, were sown December 25, 1981, in lines 30 cm apart. The plants were thinned 21 days after emergence to maintain an intrarow spacing of 5 cm. Rainfall received during the crop season was 100.50 mm.

Table 1. Effect of levels of irrigation on yield and yield attributes of a few soybean varieties

Treatment	Number of pods/plant	100-seed weight (g)	grain yield (q/ha)
Irrigation (I)			
Pre-sowing irrigation (I <sub>1</sub> )	11.25	11.20	5.10
Pre-sowing irrigation followed by one irrigation at 40 mm CPE (I.	17.83 <sub>2</sub> )	13.28	8.59
Pre-sowing irrigation followed by two irrigation at 40 and 75 mm CPE $(I_3)$	27.58	14.79	11.96
CD 5%	3.74	0.77	0.39
Varieties (V)			
DS 73-16 (V <sub>1</sub> )	30.22	13.81	12.75
JS-2 (V <sub>2</sub> )	14.22	13.61	6.44
JS 72-375 (V <sub>3</sub> )	15.33	12.62	7.68
PK-271 (V <sub>4</sub> )	15.77	12.32	7.37
CD 5%	2.25	0.67	0.65

Table 2. Number of pods/plant and grain yield (q/ha) as influenced by levels of irrigation and soybean varieties

Irrigatio	ns Nu	mber of p	pods/pla	nt	Grain yield (q/ha)				
Varieties	· · <sup>I</sup> 1	12	<sup>1</sup> 3	Mean	<sup>I</sup> 1	I <sub>2</sub>	<sup>1</sup> 3	Mean	
DS 73-16	17.67	27.67	45.33	30.22	7.40	11.90	18.96	12.75	
JS-2	7.67	15.33	19.67	14.22	2.93	6.43	9.96	6.44	
JS-72-375	9.00	14.00	23.00	15.33	5.47	8.40	9.13	7.68	
PK 271	10.67	14.33	22.33	15.78	4.63	7.67	9.80	7.37	
Mean	11.25	17.83	27.58		5.10	8.59	11.96		
	CD 5%				CD 5%				
V within I	3.90				1.11				
I within V	5.00				1.03				

An appraisal of the data in Table 1 revealed that the highest grain yield of 11.96 q/ha was associated with the treatment pre-sowing irrigation followed by two irrigations at 40 and 75 mm CPE (I $_3$ ) and was followed by I $_2$  and I $_1$  in decreasing order. The increase in yield in I $_3$  and I $_2$  over I $_1$  was 61.0 and 43.8%, respectively. Beneficial effect of supplementary irrigation was due to significant improvement in number of pods/plant as well as 100-seed weight.

The variety DS 73-16 produced significantly higher grain yield (12.75 q/ha) than rest of the varieties. The varieties JS 72-375 and PK-271 were at par in respect to grain yield and the variety JS-2 produced the lowest yield (6.44 q/ha). Higher grain yield associated with the variety DS 73-16 was due to significantly higher number of pods/plant (30.22) and 100-seed weight (13.81 g).

Each supplementary irrigation caused the grain yield to increase significantly over the preceding one in all the varieties (Table 2). The variety DS 73-16 maintained its superiority over the others in each level of irrigation and produced the highest grain yield of 18.96 q/ha with pre-sowing irrigation followed by two irrigations at 40 and 75 mm CPE. Similar trend was observed for the number of pods/plant.

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### 6) Effect of dates of planting on five soybean varieties

Assam is one of the seven states comprising the North Eastern region of India. The plains of the Brahmaputra Valley lie between 24° to 28° N Latitude, 90° to 96° East Longitude and its elevation (above M.S.L.) ranges from 35 m (Dhubri) to 106 m (Dibrugarh). Soybean was introduced in Assam in 1975 through the Soybean Research Project sponsored by the Indian Council of Agriculture Research, New Delhi. Since then, its yield potential has been found high; the average grain yield ranging from 2.5 to 3.0 t/ha with the highest reaching 4.5 t/ha. Soybean is a determinant crop. It was, therefore, felt necessary to identify the best sowing time for the varieties suitable for the area. A field trial was conducted at Assam Agricultural University Farm, Jorhat, during rainy season of 1980 (July to October) on sandy loam soil, to find out the optimum dates of sowing of the recommended varieties of soybean.

The experiment was laid out in a randomized block design with three replications. The size of the individual plot was 4 m x 3.15 m and all plots received basal application of N,  $P_2O_5$  and  $K_2O$  @ 20 kg, 80 kg and 60 kg per hectare, respectively. The seeds were treated with *Rhizobium* culture @ 5 g/ha of seed at the time of sowing and were sown in rows 45 cm apart. The experimental plots were kept free from weeds throughout the growing season by hand weeding.

Data on grain yield, weight of 100-seeds and plant height are presented in Table 1. The grain yield of soybean was significantly influenced by different dates of sowing. The earliest date of sowing (May 16) recorded significantly the highest grain yield (22.70 g/ha) compared with the other dates of sowing. Delay in sowing beyond May 16 reduced grain yield significantly. The higher grain yield obtained due to early sowing on May 16 may be due to varietal behavior or efficient utilization of soil moisture. The reduction in grain yield in later dates of planting may be accounted for by low rainfall at the time of pod formation and all other varieties were short duration excepting 'Bragg' and UPSM-19. Higher yields of soybean due to early planting (May and early June) as compared with late planting (beyond June last) were also reported by Maley and Sharma (1973), Lokras and Tiwari (1977) and Lokras (1980).

One-hundred-seed weight and plant weight differed significantly for planting dates. Crops planted on earlier dates were vigorous in growth as would be evident from the data on plant height as compared with late planting.

Varieties showed perceptible variations in respect to growth (plant height) and 100-grain weight (Table 1) which influenced the grain yield of the varieties. Variety Bragg gave the highest grain yield (15.52 q/ha) which was at par with UPSM-19 (14.80 q/ha) and significantly outyielded the other varieties. These two varieties had heavier seed weight and plant height which may be the possible causes for higher yield. A measure of relative productive efficiency for the varieties was also worked out in terms of grain yield per day per hectare. The per day per hectare yields were more in JS-72-1 (14.67 kg) and JS-2 (14.61 kg) than the other three varieties. In an intensive multiple cropping system where both time and space are equally important, these

Table 1. Effect of dates of planting on yield attributes and yield (q/ha) of soybean varieties

Treatments	Plant height (cm)	100-grain weight (g)	Days to maturity	Grain yield (q/ha)	Grain yield (kg/day/ha)
Date of planting	(D)				
May 16	76.94	14.18	111.20	22.70	20.41
June 30	88.74	15.20	108.87	19.95	18.32
July 22	74.81	14.39	104.00	10.30	9.90
August 20	51.54	13.62	97.80	2.96	3.03
SED (D. Means)	2.86	0.22	0.56	0.95	
C.D. (0.05)	5.77	0.44	1.13	1.92	
Varieties (V)					
Bragg	98.33	15.32	122.25	15.52	12.53
JS-2	45.55	13.55	90.00	13.15	14.61
JS-72-1	87.10	14.48	92.25	13.54	14.67
JS 72-375	51.56	13.44	96.08	12.89	13.42
UPSM-19	115.54	14.95	126.75	14.80	11.68
SED (V. Mean)	3.19	0.24	0.62	1.06	
C.D. (0.05)	6.45	0.49	1.26	2.14	

Interaction effects of date of planting and variety on yield attributes and yield of soybean Table 2.

Dotto Of		Plant height (cm)	ght (cm)			Days to	Days to maturity		100	100-seed weight (g)	eight (g	
planting (D) Variety (V)	May 16	June 30	July 22	August 20	May 16	June 30	July 22	August 20	May 16	June 30	July 22	August 20
Bragg	130.13	127.30	82.00	53.87	135.00	130.33	115.67	108.00	15.58	15.56	15.39	14.74
JS-2	55.53	42.97	51.63	32.07	91.00	00.06	00.06	89.00	13,53	13.65	13.32	13.68
JS 72-1	121.57	19.46	81.20	50.97	95.00	92.00	92.00	90.00	13,10	16.17	14.60	14.06
JS 72-375	71.73	41.03	46.93	46.53	98.00	97.00	97.33	92.00	13.50	15.54	13.64	11.07
UPSM-19	137.87	137.73	112,30	74.27	137.00	135.00	135.00 125.00	110.00	15.18	15.08	14.98	14.57
SEd (Dx V)		. 6	6.38				1.25			0	0.49	
C.D. (0.05)		12.91	91			2.	2.52			0	0.97	

two varieties would be considered useful. Differential responses of dates of planting were found with the varieties in respect to plant height dates to maturity and 100-seed weight which is presented in Table 2.

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100

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### 1) Phenotypic stability of soybean genotypes for field germination

Germination of seed is a limiting factor in successful cultivation of soybean in northeast India. Information regarding genotype x environment (GE) interaction and phenotypic stability of different genotypes for field germination percentage is of considerable importance in order to isolate the most stable genotypes with uniform germination potential over variable environments. The present study, therefore, was designed to study the phenotypic stability of ten soybean genotypes grown in six different environments.

Materials and methods: The materials included in the present investigation are ten soybean genotypes, viz., 'Bragg', 'JS2', 'PK369', 'Kalitur', 'JS72-1', 'PK71-21', 'PK327', 'JS72-375', 'PK409' and 'DS73-16'. These genotypes were sown in six sowing dates — three in winter and three in spring season and each sowing represented an environment:  $E_1(28.12.81)$ ,  $E_2(28.1.82)$ ,  $E_3(3.3.82)$ ,  $E_4(17.7.82)$ ,  $E_5(13.8.82)$  and  $E_6(20.9.82)$ .

The whole experiment was laid out in the Research Farm of the Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat, Assam, India, following the randomized block design with three replications separately for each environment. The spacing between plants was 10 cm and between rows 45 cm. Five rows were allotted per plant, each row measuring 2m. Observations on field germination were recorded by counting the number of germinated seeds in each plot excluding the border rows and the counts of each plot were converted to percent germination. The mean data of germination percentages so obtained were analyzed following the Eberhart and Russell (1966) model in order to assess the phenotypic stability of soybean genotypes for field germination. The model provides three stability parameters, viz., mean  $(m_i)$ , regression coefficient  $(b_i)$  of genotypic mean on environmental indices and deviation mean square  $(S^2\vec{d}_i)$ . In addition, phenotypic index  $(P_i)$  was computed for each genotype as the difference of the mean of the ith genotype from grand mean.

<u>Discussion</u>: The analysis of variance in individual environments for field germination of soybean genotypes is presented in Table 1. In all the environments, the variance was found to be significant except  $\mathbf{E}_6$ .

The significant genotypic variances in almost all the environments indicated that the genotypes have sufficient genetic variation for field germination. Of course, the genotypes did not show significant effect of the different factors of E<sub>6</sub> on the expression of genetic variability in respect of field germination which was more sensitive to this environment. But the pooled analyses over all the environments clearly indicated sufficient genetic variation for this character.

The pooled analysis of variance (Table 2) also revealed significant genotypic difference for field germination. The additive environmental effect was of considerable magnitude as indicated by significant environment (linear) component. The genotypes were observed to interact significantly with the additive environmental variability. Further, both GE interaction (linear) and pooled deviation were also found to be significant indicating the contribution of both linear and nonlinear components towards the GE interaction variance.

Table 1. Analyses of variance in individual environments

Source	Degree of			Mean so	<sub>[uares</sub>		
Boarce	freedom	E <sub>1</sub>	E <sub>2</sub>	E <sub>3</sub>	E <sub>4</sub>	E <sub>5</sub>	E <sub>6</sub>
Replication	n 2	259 <b>.7</b> 2 **	358.26 **	6.91 **	141.44 **	0.96 **	4.69
Genotype	9	139.89	474.23	251.99	406.87	893.53	81.67
Error	18	27.79	22.87	42.78	41.25	18.39	3.51

<sup>\*\*</sup>P < 0.01.

Table 2. Pooled analysis of variance for genotype x environment interaction

Source	Degree of freedom	Mean square
		**
Genotype (G)	9	211.59
Genotype x environment (GE) interaction	50	298.01 **
Environment (linear)	1	9789.64 **
GE interaction (linear)	9	97.01 **
Pooled deviation	40	105.95
Bragg	4	7.06 **
JS 2	4	272.05 **
PK 369	4	74.62 **
Kalitur	4	19.51 **
JS 72-1	4	119.50 **
PK 72-21	4	36.03 **
PK 327	4	349.20 **
JS 72-375	4	84.33 **
РК 409	4	51.60 **
DS 73-16	4	40.58
Pooled error	120	8.70

<sup>\*\*</sup>P < 0.01.

From Table 3, genotype Kalitur was found to be with average stability as it possessed comparatively high mean performance ( $P_i = 11.86$ ), linear regression approached almost unity and low deviation mean square. The genotype Bragg exhibited high mean germination percentage ( $P_i = 6.92$ ). This genotype further exhibited high regression coefficient ( $b_i > 1$ ) and low deviation mean square. All these indicated that the genotype possessed below average stability. Thus, while Kalitur possessed the inherent capability to exhibit uniform germination potential over a wide range of environmental conditions, Bragg could be a suitable genotype in high-yielding ideal environment in which its germination percentage would be much higher than its actual potential. Although the genotype JS 2 exhibited low mean germination percentage ( $P_i = -1.73$ ) and high deviation mean square, it has low regression coefficient ( $b_i$  < 1). From linear stability point of view alone, the genotype possessed above average stability. There is sufficient evidence that the mean performance and the ability to perform consistently over variable environments are two independent characters which can be genetically manipulated (Bucio-Alanis et al., 1969; Bains, 1976). In view of this fact, the genotypes exhibiting above average stability for field germination have considerable significance from agronomic and plant breeding point of view. Smith et al. (1967) observed that the genotypes expressing average stability were influenced less by changing environments. Similar observations were reported by Verma et al. (1972).

Table 3. Stability parameters of the soybean genotypes for field germination

		Stability p	arameters	
Genotype	m	Pi	b <sub>i</sub>	$S^2d_i$
			*	
Bragg	42.18	6.92	1.41	1.63 **
JS 2	33.53	-1.73	0.49	263 <b>.</b> 36 **
PK 369	32.25	-3.01	0.97	65.93
Kalitur	47.12	11.86	1.05	28.31 **
JS 72-1	37.53	2.27	1.29	110.81
PK 71-21	36.23	-0.03	1.18	37.33 **
PK 327	33.35	-1.91	1.34	328.01 **
JS 72-375	28.65	-6.61	0.87	75.63 **
PK 409	27.33	-7.93	0.83	42.91 **
DS 73-16	34.52	-0.74	0.58	31.88

<sup>\*</sup>P < 0.05.

<sup>\*\*</sup>P < 0.01.

Summary: Information on genotype x environment (GE) interaction and phenotypic stability of the genotypes for field germination percentage is of considerable interest in order to isolate the most stable genotypes with uniform germination potential over different environments. Thus, to study the phenotypic stability of field germination of soybean, ten genotypes were grown in six environments during 1981-82 in the departmental Research Farm of the Dept. of Plant Breeding and Genetics, Assam Agricultural University, Jorhat, Assam, India. The analysis was done following the Eberhart and Russell (1966) model.

Genotypes exhibited sufficient genetic variation for field germination. The genotypes were found to interact significantly with the additive environmental variabilities. Both linear and nonlinear components of GE interaction were found to be significant for the field germination of soybean. Genotype Kalitur exhibited average stability; below average stability was exhibited by Bragg and above average stability by JS 2 for the field germination percentage.

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## 1) Potential of an off-season soybean nursery

In India, soybean is generally sown during summer (June-October). However, due to tremendous variability in climatic conditions of the country, there is a scope for growing soybean in more than one season. The efficacy of yield improvement projects could be substantially enhanced by rapid generation turnover. Theoretically, it is possible to achieve an annual turnover of three generations using a winter crop sown in late October or early November (short day conditions), followed by a spring crop in March and a normal summer crop planted in June. At the onset, we wish to make it very apparent that our objective is generation advancement only, and selection should invariably be practiced in normal season. Assuming an annual turnover of three generations, this system will allow yield testing of  $F_3$ -derived  $F_6$  families only two years after making the initial cross.

In winter, 1982, we initiated feasibility studies of an off-season nursery at Ranchi, Bihar, in the main plateau region of India, at an altitude of 635 meters. Average rainfall and temperature during this season varies from 6.9 mm to 79.5 mm and 16°C to 19°C, respectively. Four varieties, i.e., 'Birsa Soy 1', 'N-22', 'Seminal', and 'Cockerstewart', were selected for the experiments. Crop was sown on 20 December onwards at a regular interval of 10 days. The data for various characters are presented in Table 1. As compared with the normal season, the germination was substantially lower, onset of blooming and maturity were considerably delayed. Overnight presoaking of seeds prior to sowing significantly improved germination. Plants, in general, were shortstatured as compared with normal season. Pollen fertility was reduced, but female fertility (expressed as natural seed set as a percent of ovule number) was normal. Due to very delayed maturity under winter-sown conditions, theoretical target of three generations per year may be difficult to achieve. However, at a limited scale, the winter crop may be sown immediately after harvesting the summer crop in mid-October. In addition, some hormonal treatments could be thought of to achieve an early onset of maturity. It will be interesting to evaluate sowing schedule for spring sowing in March, as the onset of high temperature condition during May and June might hasten the maturity.

Table 1. Average performance of four soybean cultivars in the off-season nursery

			Character		
Date of sowing	Germination (%)	Days to bloom	Days to maturity	Pollen fertility (%)	Female fertility (%)
20.11.1982	48	80	148	67	73
30.11.1982	57	75	138	72	66
10.12.1982	46	75	146	59	69
20.12.1982	30	67	135	60	67
30.12.1981	26	70	139	63	65
10.1.1983	38	64	138	67	66
20.1.1983	45	70	136	60	70
Summer crop	67	45	110		

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### 2) Mutation breeding research in soybean in India//

Systematic mutation breeding research in India was started at Ranchi Agricultural College, Kanke, Bihar, India, around 1971 by Haque and his co-workers. Choudhary (1972) studied induced polygenic variability in the R-II generation in the variety of 'Sepaya Black' of soybean. Choudhary (1972) reported that 10 Kr radiation treatment of gamma rays was effective in shifting the mean values in positive direction for various quantitative characters including seed yield. During the course of investigation, one spontaneous mutant was observed in the variety Sepaya Black and further selection in the spontaneous mutant has led to the release of a new variety of soybean known as 'Birsa Soybean-1'. This variety has been recommended for release for the plateau region of Bihar state in India.

In order to change the black color of Birsa Soybean-1, seeds were treated with different doses of  $\gamma$ -rays at FCI Sindri (Bihar) in the year 1979. In Ml generation, superior plants were selected and their progenies were raised in 1980. From the M2 generation, promising progenies, as well as single plants, were selected and in 1981, the M3 generation was raised.

Progeny of a yellow seeded 50-Kr M2 plant in M3 gave very promising and interesting plant types. Sixteen of these were selected and the characteristics of these promising plants are as follows.

Table 1. Characteristics of M3 segregation of Birsa Soy-1 of a yellow-seeded mutant line of Kr 50

Segregate plant no.	Height (cm) l	No. oranches	No. pods	No. seeds	Seed color	Hilum color	Seed weight/ plant (g)	Test-weight (100 seed weight) (g)
1	54.5	6	250	517	Y	Black	59.18	11.45
2	43.5	6	110	158	Dull brown	White	18.04	11.40
3	46.0	9	149	260	В	В	50.70	19.50
4	40.0	11	114	238	В	В	29.27	12.30
5	43.0	11	148	226	Y	В	30.74	13.60
6	45.0	5	125	227	Y	В	23.80	10.49
7	40.0	9	112	216	Y	В	25.12	11.63
8	32.0	10	111	221	Y	В	31.03	14.04
9	33.0	13	80	164	Y	Dull	18.63	11.36
						brown		
10	33.0	6	87	175	Y	В	22.76	13.00
11	30.0	11	115	217	Y	В	21.67	9.90
12	36.0	8	42	73	В	В	6.82	9.34
13	35.0	9	78	118	Y	Dull brown	11.60	9.83
14	119.0	9	167	357	В	В	51.90	14.54
15	80.0	9	338	558	Y	Dul1	54.99	9.85
						brown		
16	79.0	7	259	405	Y	В	56.09	13.84
Birsa Soy- (parent va		5.4	44.5	115	В	White	10.42	13.33

The changes in color of the Birsa Soybean-1 from black to yellow, dull brown, brown have been established. Further screening of the material for yield potential is in progress.

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# 1) Induced parthenogenesis in soybean

A field of research currently gaining much interest is the irradiated-pollen-induced ovule transformation in plants. The interest lies mainly in the possibility of modifying the plant genome in a quicker and more economical way. The successful demonstration of such gene transfers have been achieved in Nicotiana (Pandey, 1975; Jinks et al., 1981), Brassica (Banga et al., 1983) and Lycopersicum (Zamir, 1983) for both qualitative and metric traits. This technique is based on the resistance of pollen-grain function to ionizing radiation damage. Heavily irradiated (>100 Krad) are still able to germinate, grow down the style, and effect pseudofertilization leading to haploid or diploid parthenogenesis. The pollen tubes deliver their fragmented DNA to the embryo sacs. The DNA fragments delivered in this manner can be incorporated in the egg nucleus (Pandey, 1980).

Two different strains of soybean (Glycine max [L.] Merr.) with the homozygous genotype, one for white flower (M-4) and another for violet flower (M-9), were utilized in this study. The pollen from M-9 was irradiated with 0, 35, and 50 Krad of gamma rays and used to pollinate flowers of M-4. Controlled emasculations without pollination failed to set any seed, overruling the theory of spontaneous parthenogenesis. Seed set was very small (<2%) for 50 Krad dose, while it was up to 5% for 35 Krad. Mostly small and shrivelled seeds were obtained. It is suspected that, at higher dose (50 Krad), the majority of seed set were haploids. Preliminary studies indicate them to be parthenogenates. Detailed investigations are underway to demonstrate the transfer of paternal characters in a largely maternal background.

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## 1) Breeding for resistance to yellow mosaic virus through interspecific hybridization in soybean

Yellow mosaic is one of the major diseases of soybean in the northern part of India and in Bangladesh and Sri Lanka. It is more serious in the Tarai region of Uttar Pradesh and has caused a setback to its cultivation. The virus is transmitted through white fly (Bemisia tabaci Genn). Resistance to this disease was reported from this breeding program by Singh et al. (1974) in PI 171443 (UPSM-534) and Glycine formosana. Resistance in UPSM-534 is due to two recessive gene pairs (Singh and Malick, 1978) and in Glycine formosana, probably due to one dominant gene. These two donors are frequently being used in our ongoing soybean breeding program. Here, we wish to examine the prospects of new breeding lines having yellow mosaic resistance from Glycine formosana.

Glycine formosana is a typical wild-looking soybean. It was introduced by B. B. Singh, ex-soybean breeder of this university through the courtesy of K. L. Chan, Taiwan Agricultural Research Institute, Taipei. It has very narrow leaves and indeterminate growth habit. It matures in about 130 days and pods shatter easily. It is resistant to yellow mosaic and susceptible to bacterial pustules. It can be crossed easily with cultivated soybeans (Singh et al., 1974). Glycine formosana was crossed with 'Bragg' (susceptible to yellow mosaic and resistant to bacterial pustules). The  $F_1$  was back-crossed with Bragg and the BC<sub>1</sub> progenies were handled through pedigree method of breeding, selecting for yellow mosaic resistance and other desirable economic traits. Straight  $F_1$ s advanced to  $F_2$  and  $F_3$  failed to generate agronomically superior lines and were rejected. The performance of yellow mosaic resistant lines developed through this program is given in Table 1. In 1980 evaluation, all the six breeding lines demonstrated resistance to yellow mosaic and outyielded Bragg (check), although the yield difference was not significant. evaluation, all 19 lines outyielded Bragg. PK-502, PK-505, PK-507, PK-508, PK-510, PK-520 and PK-522 gave significantly higher yield than Bragg. evaluation, all the six breeding lines outyielded Bragg significantly. these newly developed soybean breeding lines derived from (Glycine formosana x Bragg) x Bragg crosses had resistance to yellow mosaic and gave better yield than Bragg.

Some of these lines (PK-486 and PK-515), when tested at various locations under all India coordinated soybean research program, gave better yield performance across the locations (AICRPS, 1983). PK-486 is in the final stage of evaluation in the coordinated elite varietal trial and is likely to be released for the northern plains of India.

Therefore, it is obvious that *Glycine formosana* can be successfully utilized in a soybean breeding program to develop varieties resistant to yellow mosaic. Only one back-cross is adequate, and further handling of BC<sub>1</sub> progenies in pedigree method was found to be satisfactory. Some of the newly derived lines from (*Glycine formosana* x Bragg) x Bragg crosses were prone to shattering and lodging. This was due to the fact that *Glycine formosana* is a shattering type and has prostrate growth habit. The stem is viny and weak. Hence, while handling segregating lines derived from crosses involving *Glycine formosana*, care has to be taken to eliminate such lines during selection.

Table 1. Yield performance of soybean lines having yellow mosaic resistance gene from  $Glycine\ formosana$ 

Year of evaluation	Line	Days to flower	Days to maturity	Plant height (cm)	Pods per plant	Seeds per pod	100-seed weight (g)	Seed yield (kg/ha)
	***************************************							
1980	PK-496		119	65	88	1.90	11.2	2100
	PK-487		119	72	84	2.15	8.1	1806
	PK-488		120	70	76	2.05	13.0	2030
	PK-489		120	71	87	1.90	12.7	1961
	PK-490	52	119	66	92	1.95	13.7	2187
	PK-491	52	119	71	78	2.00	12.0	2014
	Bragg	48	120	66	91	2.10	16.7	1875
CD 5%								N.S.
CV (%)	. <b>_</b>							21.59
1981	PK-502	46	116	70	101	2.20	9.0	2315
	PK-504	49	119	87	64	2.30	11.0	2100
	PK-505	53	118	77	79	2.40	11.8	2534
	PK-506	52	119	74	79	2.25	13.0	1961
	PK-507	49	118	79	76	2.25	11.1	2292
	PK-508	48	118	76	73	2.25	13.0	2759
	PK-509	48	117	87	104	2.15	12.3	2031
	PK-510	49	117	72	104	2.40	13.1	2483
	PK-511	47	116	92	70	2.20	10.5	1858
	PK-512	49	117	67	83	2.30	8.5	1597
	PK-514	47	117	81	99	2.25	9.2	1996
	PK-515	53	120	88	95	2.35	8.0	2170
	PK-516	49	118	87	92	2.10	14.5	2031
	PK-517	48	120	72	70	2.12	10.5	2083
	PK-518	49	118	104	94	2.10	15.3	1770
	PK-519	47	121	71	88	2.50	10.7	2535
	Bragg	45	119	69	98	2.15	15.3	1563
CD 5%								643
CV (%)								22.42

Table 1. Continued

Year of evaluation	Line	Days to flower	Days to maturity	Plant height (cm)	Pods per plant	Seeds per pod	100-seed weight (g)	Seed yield (kg/ha)
	PK-520	46	117	69	88	2.60	10.4	2674
	PK-521	46	118	71	120	2.26	10.1	2362
	PK-522	46	116	77	124	2.30	10.4	2622
	Bragg	46	117	70	86	2.20	15.0	1753
CD 5%								619
CV (%)								19.83
1982	PK-699	52	118	62	54	2.30	16.9	2083
	PK-700	60	129	63	49	2.30	12.5	2361
	PK-701	54	123	85	68	1.80	13.5	2083
	PK-702	52	117	55	76	2.20	13.9	2291
	PK-703	53	120	50	45	2.40	15.7	2152
	PK-704	54	120	70	49	2.20	16.2	2291
	Bragg	51	117	63	72	2.20	13.1	1388
CD 5%								479
CV (%)								18.40

NB: Bragg susceptible to yellow mosaic.

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1) Effect of temperature on fasciation characters in fasciated soybean./

Introduction: The phenomenon of fasciation in soybean as a consequence of spontaneous mutation has been known for a long time under the name of 'Keitomame' or 'Shakujo-mame'. Fasciated soybean has a peculiar character in which the stem is flattened, with a few number of branches. The flowers develop as a cluster at the top of the stem and most pods accumulate at that part (Takahashi and Fukuyama, 1919; Takagi, 1929). It has been reported that stem fasciation is controlled by a single recessive gene, f (Takagi, 1929).

From our investigation on morphology of fasciated soybeans, the fasciation characters have been classified into four characters including 1) the leaf development on main stem, 2) stem fasciation (flattened stem), 3) a scarcely branching habit and 4) pod setting.

Generally, it has been known that temperature has an effect on many growth processes of the soybean plant and affect the adaptability of variety. The objective of this study was to investigate the effect of temperature on fasciation characters in fasciated soybean.

Materials and methods: The experiment was conducted in 1981 and 1982 at the Biotron Institute of Kyushu University, Fukuoka, Japan. Three constant controlled temperatures of 20°, 25° and 30°C under natural sunlight and the natural daily fluctuating temperature were used in this study.

Four varieties of fasciated soybean consisting of Karikou 259, Keitodaizu, Shakujo (Bb1132) and T 173 were used. Each treatment consisted of two plants. The experiment was conducted from May 20 to the end of September in controlled room, then the plants were allowed to grow till maturity in natural conditions. Since the results of the two years study were nearly similar, only the observations in 1982 are presented.

Results and discussion: All fasciated soybeans frequently developed one to four leaves on the same day in all temperatures, though their frequencies were different among varieties and temperatures. It is obvious that leaf emergence of fasciated soybeans differed from normal soybean in all treatments. The day interval of leaf emergence on main stem in fasciated soybeans were less than the normal soybean.

The number of leaves on the main stem in fasciated soybeans was greater than normal soybean in all temperatures. However, within temperature treatment, the duration of leaf development were similar in two soybean types (Table 1). The number of leaves was lowest at 25°C in most of the varieties except Karikou 259, which gave nearly the same number of leaves at the three temperatures studied.

Leaf arrangements were similar in all treatments. Leaves on main stem were arranged in inconsistent arrangement surrounding the stem.

Table 1. Effects of temperature on leaf development on the main stem in fasciated soybeans

Character	Temperature °C	Karikou 259	Keito- daizu	Shakujo (Bb1132)	Т 173	Hime−daizu*
Number of leaves on the main stem	20	58	63	56	67	17
	25	58	47	87	41	16
	30	09	20	59	43	21
	Control	78	09	61	39	18
Duration of leaf development	20	79	81	81	92	92
on the main stem (days)	25	51	28	58	58	51
	30	51	28	28	51	61
	Control	89	71	63	58	99

\*Normal soybean.

Stem fasciation was evident in all temperatures (Table 2). However, varieties exhibited different degrees of stem fasciation at those temperature treatments. All varieties gave a low degree of stem fasciation at 30°C except Shakujo (Bbl132). We speculated that the decrease in stem fasciation at 30°C might be due to the increase in the internode length. Analysis of variance of temperature, variety, and variety x temperature interaction for stem fasciation showed no significant differences. We concluded that temperature did not have any effect on degree of stem fasciation. Albertsen et al. (1983) reported that increased leaf number on main stem depended on the expression of fasciation. We observed that leaf number did not relate to the expression of stem fasciation.

All fasciated soybean tested varieties exhibited a low degree of branching in all conditions (Table 2). All evidence for branch number at those temperature treatments indicated that temperature has little effect on branch producing in fasciated soybeans. This indicates that genetic effect on branching is considerably stable, irrespectively of temperature.

Table 2. Stem fasciation and number of branches per plant in fasciated soybeans

Variety	St	em fasci	ation (	cm) <sup>a</sup>		Brancl	hes/plai	nt
Temperature °C	20	25	30	Control	20	25	30	Control
Karikou 259	2.01	2.31	1.93	2.81	1.5	0.0	0.0	3.0
Keito-daizu	2.57	2.35	1.90	2.53	0.5	0.5	1.0	1.5
Shakujo (Bb1132)	1.93	2.00	2.02	3.36	0.5	0.0	2.0	1.0
Т 173	1.91	1.98	1.42	2.04	1.5	0.0	0.0	3.5
Hime-daizu*					6.5	7.0	6.0	10.0

<sup>&</sup>lt;sup>a</sup>Stem fasciation was measured at the maximum broad part.

Pod setting was affected by temperature, especially at the stem tip (Table 3). One of the remarkable characteristics in fasciated soybean is that most of the pods developed at the stem tip in a cluster. Karikou 259 gave a similar number of pod percentage in all conditions. This result suggested that Karikou 259 has a good adaptability for temperature effect. Keito-daizu and Shakujo (Bb1132) have a good performance for this character at 20°C and 25°C, while T 173 produced most at 25°C and 30°C. Number of pods per plant increased with increased temperature except Shakujo (Bb1132) which showed maximum pod numbers at 25°C as well as Hime-daizu, normal soybean.

The number of pods per plant and pod setting at the stem tip did not relate to the degree of stem fasciation. We noted that most of the fasciated soybean tested varieties are the determinate type, except T 173, which seems to be the indeterminate type. Fasciated soybeans can exhibit the fasciation characters in all temperatures studied.

The results suggest that there are wide variation and interesting traits in fasciated soybean to be studied further.

<sup>\*</sup>Normal soybean.

Table 3. Number of pods per plant and percent of pod at the 10 cm length from the stem tip in fasciated soybeans

Variety		Pod	s/plant		Pod	percent	age at	the top
Temperature °C	20	25	30	Control	20	25	30	Control
Karikou 259	30	62	64	71	52	63	49	54
Keito-daizu	56	82	112	118	66	51	20	58
Shakujo(Bb1132)	51	80	69	110	83	31	22	62
Т 173	10	88	113	105	16	58	38	45
Hime-daizu*	101	165	137	241	11	8	30	23

\*Normal soybean.

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1) Identification and evaluation for mutation of agricultural characters in soybean

In genetic and breeding work for soybean, it is essential to establish a variety adaptable to the climatic conditions of Poland. Induction of mutations is likely to broaden the variation range and help select traits favorable from the breeders' viewpoint. An attempt to induce mutations was made on material genetically differentiated. Careful attention was paid to the prospect of selecting mutant variation in the M2 and M3, relating to the length of growing period, morphotype and yield capacity.

Materials and methods: Seeds of 14 soybean varieties were treated with various mutagenic agents in three combinations: (1) radiation with 10 kR; (2) radiation with 10 kR in addition to  $10^{-3} \text{M NaN}_3$  solution against 0.1M phosphate buffer at pH 3 for 3 hr; (3) radiation with 10 kR, presoaking in  $\text{H}_2\text{O}_2$  for 3 hr, treatment with  $\text{NaN}_3$  solution. In the  $\text{M}_1$ , the number of well-developed seeds recorded for the three combinations were as follows: 2766, 875, and 173, respectively. Totally, 3814 seeds were obtained from gamma radiation combinations (Table 1). Seed of 7 varieties were treated with chemicals for 18 hr. From these combinations, 4112 seeds were obtained in the  $\text{M}_2$  (Table 1). Seed of the  $\text{M}_2$  generation and  $\text{M}_3$  lines, derived the following year from the  $\text{M}_2$  mutants, were grown in the field in rows spaced 10 x 30 cm. Observations were made on developmental stages, morphological traits, and yield capacity of single plants.

Results: A comparison between controls and Mo plants revealed conspicuous differences in the emergence rate and number of emerging plants. The Mo plants emerged approximately two weeks later than the controls. The highest number of late-emerging plants were recorded for the varieties 'Herb 622' and 'Nordia', despite their high emergence percentage (Table 1). Poor and uneven emergence was noted for the M2 of 'Altona', 'Mazowiecka II', 'Vansoy', 'Ik-9', and 'Woronożeska'. Totally, 69.1% plants emerged from gamma radiation in the Ma and 80.7% from chemical mutagen treatment (Table 1). At the beginning of the growing period, remarkable morphological abnormalities were observed. affected primarily the onset of single leaflets at cotyledon angles and above the first pair of true leaves, atrophy of the top of main stem in plants 10-20 cm high, the size and shape of leaf-blades. Deformations of leaflets at the true leaf were observed over the whole growing period and were even visible at apical leaves. The abundance of abnormal leaves was largest in the Ma of the following varieties: Mazowiecka II, Herb 622, Altona, and Vansoy. The high number of deformations, in addition to chlorophyll aberrations, manifest an effective action of mutagens on M2 plants. All plants showing any chlorophyll aberrations were recorded. In some combinations (e.g., the M2 of Altona, IInd combination) the number of aberrations reached 43.9% (Table 1). No regularity was observed between the frequency of chlorophyll aberrations and that of induced mutations.

As far as the phenotypic traits are concerned, the uneven emergence seems to have caused the differentiated growth rate and additionally influence the

Table 1. Emergence of plants and frequency of chlorophyll aberrations and mutants in  $\mathbf{M}_2$  generation

Variety/ combination of treatment	No. grown seeds	Emerg No.	ence %	Frequench chlorol	phy11	Frequ o muta	f
	00000	1.00	V	No.	%	No.	%
Altona	50	50	100.0				
I	170	111	65.3	44	39.6	4	3.6
II	88	57	64.8	25	43.9	_	_
III	141	131	92.9	36	27.5	7	5.3
American Yellow	50	36	72.0				
I	147	91	61.9	18	19.8	6	6.6
Amurska	50	44	88.0				
I	373	304	81.5	46	15.1	19	6.2
II	85	69	81.2	11	15.9	4	5.8
Fiskeby V	50	37	74.0				
I	183	140	76.5	31	22.1	9	6.4
II	91	74	81.3	15	20.3	1	1.3
III	32	31	96.9	10	32.3	3	9.7
Flambeau	50	40	80.0				
I	282	246	87.2	61	24.8	12	4.9
II	158	119	75.3	47	39.5	4	3.4
Mazowiecka II	50	43	86.0				
I	644	414	64.3	48	11.6	19	4.6
II	78	52	66.7	15	28.8	-	-
Vansoy	50	43	86.0				
I 	967	551	57.0	78	14.2	10	1.8
II	375	245	65.3	26	10.6	2	0.8
Total from							
combinations	3814	2635	69.1	511	19.4	100	3.8
after radiation							
Bydgoska	50	35	70.0				
0.025% MMS	402	325	80.8	42	12.9	12	3.7
Herb 622	50	40	80.0				
0.025% EES	984	756	76.8	59	7.8	14	1.8
IK-8	50	41	82.0				
0.025% MMS	198	155	78.3	24	15.4	5	3.2
IK <b>-</b> 9	50	42	84.0				
0.025% EMS	296	168	56.7	29	17.3	9	5.3
-				-		_	

Table 1. Continued

Variety/ combination of treatment	No. grown seeds	Emerg No.	ence %	Freque chloro aberra	phy11	(	uency of ants
				No.	%	No.	%
Mari-czau-vida 0.015% NMU	50 592	45 504	90.0 85.1	176	34.9	5	1.0
Nordia 0.025% NMU	50 1268	44 1161	88.0 91.6	81	7.0	9	0.8
Woronożeska 0.025% IPMS	50 372	41 248	82.0 66.7	32	12.9	9	3.6
Total from combinations with EMS and derivatives and NMU	4112	3317	80.7	443	13.4	63	1.9

variation in developmental stages and length of growing period, particularly in Amurska, Mazowiecka II, Vansoy, and IK-8. Therefore, it was difficult to assay the extent to which abnormalities in M2 plants were of hereditary character or resulted from physiological action of mutagens on germination and development of these plants. There are different opinions on induction of mutations in soybeans for performance traits, such as shortening of growing period, in addition to increased yield and changeability of morphological characters (Johnson, 1959; Džosan et al., 1974; Ala, 1976; Siczkar, 1981). In the M<sub>2</sub> generation, a conspicuous range of variation was noted for these traits (Table 2). Hence, a good chance was offered to select for mutants prospective for crossing programs. On the basis of observations throughout the growing period and biometric measurements taken after harvest, a number of plants were selected, initially assumed as mutants. The criteria for selection were: distinct alterations in the length of growing period, seed yield, and morphological traits. As a result,  $163\ M_2$  plants were selected (3.8 and 1.9% of plants emerged) for further examination in the M<sub>2</sub>. Due to high damage by diseases, var. Mari-czau-wida was excluded from the study. No differences between Mari-czau-wida was excluded from the study. and control plants were noted in the emergence rate and number of emerging plants. Also, no single mutant line showed shortening of the phase from planting to emergence. The plants emerged some 19-20 days after sowing. During the growing period, observations were made of atypically shaped, wrinkled, and ill-colored leaflets. Also, records were taken of differences between lines and controls with respect to growth rate, plant habit, and color intensity (e.g., at the age of three weeks, plants of three mutant lines derived from Fiskeby V were 20.0 cm higher than the controls). On the other hand, the  $M_2$ plants of 'Herb 622' were observed to have distinctly slower growth rate. These differences were recorded throughout the growing period. A single line of the M<sub>2</sub> of Fiskeby V exhibited severe chlorosis resulting in necrosis of 33 from 45 plants examined at seedling stage (73.3%). The highest variation in the M<sub>2</sub>, as compared with the controls, was recorded for the length of flowering and growing period and seed yield per plant. In the present study, flowering occurred in dry weather, reported to determine seed yield at this stage of

Differences between  $\mathrm{M}_2$  plants and initial varieties in the length of growing period, morphological traits and yielding capacity Table 2.

Variety M <sub>2</sub> generation	Length of flowering period	Length of growing period	Height of plants (cm)	No. of lateral branches	No. of pods up to 15 cm	No. of seeds
Altona	25.0 +7.0	142.0 -7+16.0	62.3 -40.3+24.0	1.3	6.1 -6.1+5.9	46.1 -45.1+59.0
American Yellow	20.0	154.0	70.4 -17.0+18.0	3.7	5.8	49.9 -44.9+108.1
Amurska	20.0 +21.0	154.0 -10.0+23.0	103.0 -57.0+37.0	3.2 -3.2+3.8	0.1 +11.9	54.1 -53.1+219.0
Fiskeby V	16.0	128.0 -9+15.0	41.3 -24.0+17.0	1.8 -1.8+6.2	10.3 -9.3+17.7	35.5 -34.5+54.3
Flambeau	22.0	156.0	70.9 -24.0+32.1	2.9	2.3 -2.3+10.7	36.6 -35.6+136.4
Mazowiecka II	21.0 +18.0	147.0 -7.0+16.0	59.7 -42.7+17.3	3.8 -3.8+3.2	12.3 -12.3+27.7	93.9 -92.9+134.1
Vansoy	33.0 -4.0+18.0	153.0 +24.0	87.1 -35.1+42.9	4.3 -4.3+4.8	0.4	52.0 -51.0+183.0
Bydgoska	30.0	147.0	69.4 -32.4+11.1	3.8 -3.8+2.2	5.2 -5.2+18.8	28.4 -26.4+110.6
Herb 622	26.0	148.0 -12.0	57.3 -38.8+29.7	3.7	1.9 -1.9+10.1	33.0 -32.0+147.0
IK-8	30.0+4.0	150.0 -10.0+12.0	69.6 -31.6+45.4	2.4 -2.4+4.6	1.1	31.2 -30.2+156.8
IK-9	19.0 +14.0	148.0 -6.0	58.6 -35.6+21.4	3.1	2.5 -2.5+9.5	28.9 -27.9+63.1
Mari-czau-vida	23.0	142.0	47.8 -18.8+28.2	1.9	4.9 -4.9+9.1	27.5 -24.5+62.5
Nordia	25.0 +5.0	140.0 +7.0	50.5 -25.5+25.0	2.4	5.9 5.9+11.1	24.2 -22.2+68.8
Woronożeska	19.0	142.0	52.3 -20.3+13.7	4.0	7.0	45.9

Table 3.	Characteristics	of	selected	mutants	of.	$M_3$	generation
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Variety/ Combination treatment	Traits differing mutants from the initial variety
Altona	Onset of flowering belated by 5 days; length of flowering period shortened by 3 days.
in kr	Growing period the same as Altona. Plants higher by 10.3cm. First pod set higher. Seed yield higher by 13.5%
Fiskeby V	End of flowering 6 days later growing period 5 days longer than Fiskeby. Plants higher by 7.1cm; first pod set higher by 2.1cm. Seed yield higher by 10.0%
10 kR + H <sub>2</sub> O <sub>2</sub> + NaN <sub>3</sub>	The same type of mutants within three mutant lines. On-set of flowering 7 days later and end of flowering 3 days later than Fiskeby. Growing period longer by 3 days. Plants higher by 15.7cm; first pod set higher by 3.2cm. Seed yield lower by 30.7%
Amurska	Flowering period longer by 9 days; growing period the same as Amurska. Plants lower by 8.3cm; first pod set 4.1cm lower, more lateral branches. No. of seeds higher by 28.6%; seeds markedly smaller in size; 100-seed weigh lower by 2.6g
10 kR + NaN <sub>3</sub>	Onset of flowering belated by 15 days; flowering period shorter than Amurska by 7 days. Growing period the same as controls. Plants lower by 5.9cm. Seed yield higher by 14.0%; seeds large and plump
Mazowiecka II 10 kR	Mutant lines showing very wide variation in the length of flowering and growing periods. Within three mutant lines growing period longer by 12 days. Plants higher by approx 10.6cm. Seed yield higher by 46.0%
	Seven mutant lines with growing period and variation range similar to Mazowiecka II. Also, seed yield higher and ranging from 11.0 to 23.0%. One mutant line exhibited the same growing period and plant height as the control but seed yield higher by 43.0%
	Two mutant lines with growing period shorter than the control by 7 days. Plant height lower by approx. 10.0%. Seed yield lower by 24.5%.
	One line with the same growing period as the control; plant height lower by 10.0cm and seed yield lower by 25.00 One mutant line higher by approx. 6.7cm; no differences in growing length and yielding capacity
Herb 622	Three mutant lines with growing period shorter by 15-25
0.025% EES	days. Height of two lines lower by approx. $11.0\text{cm}$ and seed yield lower by $10.0\%$ . The highest decrease in seed yield ( $41.0\%$ ) was noted for the earliest mutant line whose plants were lower by $33.3\text{cm}$
	Growing period and seed yield the same as controls; plant height lower by 24.9%

Table 4. Frequency of selected mutant lines in  $M_3$  generation

Variety/	No. of	% selec	cted M lines ag	ainst:
combination of treatment	selected M <sub>3</sub> lines	M3 line of a given variety	M <sub>3</sub> line of the same of tre	
Altona				
I	1	9.1	25.0	0.9
Amurska	2	8.7		
I	1	4.3	5.3	0.3
II	1	4.3	25.0	1.4
Fiskeby V		30.8		
I	1	7.7	11.1	0.7
III	3	23.1	100.0	9.7
Mazowiecka II				
I	11	57.9	57.9	2.7
Bydgoska 0.025% MMS		8.3	8.3	0.3
	3	21.4		0.4
Woronożeska 0.025% IPMS	4	44.4	44.4	1.6
TOTAL	26	15.9		1.1

plant development (Mackiewicz, 1959; Szyrmer, 1971). Despite the atypical conditions, attempts were made to find out an interdependence between the length of flowering and seed yield. No data to support this hypothesis were obtained, although 14 lines of the M3 exhibited shorter or longer flowering periods than the controls. Considerable alterations in the growing period were noted for the  $M_{2}$  of Mazowiecka II, Woronożeska and Herb 622 (Table 3). Not a single mutant was found to have distinctly shorter growing period and higher seed yield. It is worth mentioning that neither mutants of the  $M_2$  and  $M_3$  of very late maturation varieties exhibited such characters (Amurská, Flambeau, Vansoy). Of interest were lines which, despite growing periods very much like the initial line, were characteristic of higher seed yield (e.g.,  $M_3$  line of Altona, Amurska, Mazowiecka II). Despite favorable phenotypic traits of  $M_3$  lines of Amurska and Mazowiecka II, their late maturation poses a serious handicap, prolonged growing likely to end in autumn and meet bad weather conditions known to produce difficulties at harvest. Most of the examined mutant lines produced from 4.1 to 5.7 g seeds per plant and had the same length of growing period and variation of morphological traits as the control plants (M $_{2}$  American Yellow, Flambeau, Vansoy, IK-8, IK-9, Nordia). Mutant lines of Vansoy had seed yield per plant reduced by half and those of American Yellow by 35.0% as compared with the initial variety. These mutants were eliminated from further studies. According to Malczeno (1970) there is little chance to improve traits of poor early mutants in the following generations. Successful selection for mutants stem from the early variety Fiskeby V, as well as for mutants with a considerably shortened growing period derived from late varieties (Herb 622, Woronożeska) is considered advantageous (Table 3). Differences in growing period, morphological traits, and yield were a good indicator of the tolerance level of mutant lines to growing conditions, as well as forming a good basis for selection of mutants.

For further studies, 26 mutant lines were selected, i.e., 15.9% of lines from the  $\rm M_3$  generation and only 1.1% of plant populations from the  $\rm M_2$  generation (Table 4). From an analysis of  $\rm M_2$  plants and one year observation of mutant lines of the  $\rm M_3$  generation, it can be assumed that the examined lines manifest a good variation, likely to contribute to crossing and selection work ahead.

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1) Evaluation of soybean germplasm for stress tolerance and biological efficiency/

(1980 Soybean Regional Report on RR-3 Project)

#### **OBJECTIVES:**

- 1. To evaluate soybean germplasm and cultivars for stress tolerance toward:
  - a. Soil Acidity
    (Tiwari, C. C., Igbokwe, P. E., Edung, S., and Russel, L.
    Alcorn State College, Mississippi)

Field experiments conducted on a silt loam soil with six cultivars under four pH ranges (5.9-6.7, 5.7-6.5, 5.3-6.0 and 5.0-5.6) indicated significant tolerance of 'Bedford' and 'Forrest' under acid soil pH ranges. The two cultivars were superior to 'Bragg', 'Braxton', 'Co-237', and 'Tracy M' in 1981 and 1982 trials. However, lowering of pH to 4.6-5.4, 4.5-5.4, 4.1-5.1, and 3.8-4.5 resulted in nullified seed formation in all the cultivars. All cultivars showed good foliage and growth, but only Bedford and Forrest showed indication of seed formation but never matured, in spite of delayed harvesting.

Results of greenhouse studies at soil pH 4.5 and 5 indicated that Bedford and Forrest contained more P, a contributing factor to superior resistance under acid conditions, compared with other cultivars (Table 1). It also seemed impractical to test for the resistance of soybean germplasm at soil pH below 5.

Findings from both the field and greenhouse, as well as laboratory trials have indicated two things that may be useful in future studies:

- 1) Acid tolerance of soybean germplasms should be evaluated at pH 5;
- 2) The P content of soybean plants harvested 38 days after germination may be helpful to the screening process of soybean germplasm.
- b. Moisture Stress
  (Singh, B. T., Fort Valley State College, Georgia)

Soybean genotypes were screened for water-stress tolerance in a split-plot experimental design with four replications. Irrigated and stress treatments were assigned main plots and the genotypes were assigned subplots. The stress water condition was induced by covering the plots with plastic. In two genotypes, FC 31649 and FC 31732, yields under irrigation and stress conditions were similar. Maximum yield reduction (57.1%) due to stress was observed in FC 31921. A summary of the results is given in Tables 2 and 3.

Table 1. P content in the different cultivars of soybean plants on two different soil pH of Memphis silt loam soil

.5 pH	5 pH
,300	1,300
,000	1,050
,050	1,250
,200	1,150
,300	1,300
,250	1,200
3 3 3	300 000 050 200 300

N.B. Bedford and Forrest, which showed the better resistance in the field, may have the ability to absorb P more efficiently, which may help in screening the soybean germplasm.

Table 2. Yield of 18 soybean genotypes under water stress and irrigation

	Yield	l (kg/ha)		Yield	(kg/ha)
Genotypes	Water stress	Irrigation	Genotypes	Water stress	Irrigation
Georgian	1,278	1,470	FC 31,921	1,119	2,033
FC 31,927	1,282	1,876	FC 31,707	1,463	1,943
Volstate	1,219	1,456	FC 30,967	1,479	2,063
FC 30,282	1,554	1,653	PI 79,861	1,145	2,028
FC 33,123	1,638	1,966	FC 31,732	1,893	1,901
PI 84,642	1,214	1,746	PI 71,570	944	1,393
FC 31,649	1,674	1,708	Creole	1,003	1,825
FC 31,622	1,441	2,260	PI 192,874	728	1,326
PI 84,967	1,138	1,709	Ransom	1,531	1,554

Table 3. ANOV for the yield of 18 genotypes under water stress and irrigation	Table 3.	ANOV for the	yield of 18	genotypes under wate	r stress and	irrigation
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Source of variation	D.F.	M.S.	F
Replication	3	1,642,709	15.50*
Water level	1	7,891,005	74.60**
Error a	3	105,766	
Genotypes	17	437,019	2.36**
Genotypes x water level	17	151,862	.82
Error b	102	185,152	

### c. Pest and Diseases

(Rangappa, M., and Benepal, P. S., Virginia State University, Virginia)

A total of 1,273 soybean germplasm lines and 39 commercial varieties were screened for natural resistance to Mexican bean beetle (MBB) under field conditions. There were 421, 314, 266, 136, and 136 germplasm and varieties from maturity groups VI, VII, VIII, IX, and X, respectively.

An average of 1,000 laboratory-reared adult MBB per day were released uniformly over all the field throughout the growing season from May until September to create an adequate MBB infestation. However, due to the prolonged drought with high temperatures and humidity in 1983, the MBB population pressure did not develop as high as compared with 1982. Nevertheless, the population was adequate to separate the test plants into tolerant and susceptible lines. Selected tolerant lines are listed in Table 4 along with the lines that will be evaluated further during 1984 growing season.

In addition to the 1,273 germplasm lines and 39 commercial varieties screened, seven cultivars from USDA, Beltsville, and 89 lines of maturity group V were selected out of 1,352 screened in 1982. Among the 89 accessions selected in 1982, fifteen accessions were observed to have less than 20% overall leaf damage over two consecutive seasons of field evaluations (Table 5). Out of seven cultivars received from USDA, Beltsville, two were observed to be the best lines (Table 5). A total of 17 resistant lines tested will be evaluated further in the greenhouse during the winter months (Table 5).

(Pacumbaba, R. P. and Sapra, V. T., Alabama A&M University, AL)

Approximately 242 improved soybean lines were screened in the greenhouse and in the field for resistance to soybean cyst nematode (SCN) races 3 & 5 during the 1983 growing season. Of this group, 21 were identified resistant and 28 were identified tolerant. Ten resistant and 12 tolerant lines were rescreened and 9 lines (AM 1074-5,

Table 4. Soybean accessions tolerant to Mexican bean beetle that had less than 20% leaf damage in 1983 season

Code	Accession	Origin
	MATURITY GROUP VI	
164	388038	Taiwan
295	416781	Japan
323	417164	Japan
	MATURITY GROUP VII	
82	171451	Japan
199	299358	Japan
	MATURITY GROUP VIII	
13	Mamotan	Delta Branch Miss., Agr. Exp. Sta.
19	Yelnanda	Pedigreed Seed Co.
25	148259	Hawaii
58	200526	Japan
64	203400	Brazil
90	206258	Philippines
102	209837	India
119	240666	Philippines
128	259539	Brazil
151	397881	India
169	324068	Rhodesia
174	341252	Brazil
207	374185	India
230	417125	Japan
	COMMERCIAL VARIETIES	
4	Cocker-237	Cocker Seed Co.
18	Ransom	VPI
26	Shore	VPI
33	Late Giant	Japan
	Black Seeded	

Table 5. Soybean accessions of Maturity Group VI tolerant to Mexican bean beetle that had less than 20% leaf damage in both 1982 and 1983 seasons

Code	Accession	Origin
35	. 81042	Japan
55	86078	Japan
80	96089	Korea
88	123440	Burma
108	159319	Korea
112	170899	South Africa
115	172902	Turkey
119	181554	Coffman Agr. Div. SCAP San Francisco
155	229339	Ministry of Agr. Tokyo
176	324924	Rhodesia
240	381670	Uganda
244	381675	Uganda
645	399095	Korea
1082	416981	Japan
1193	417419	Japan
	L-76-0132	Beltsville
	L-76-0049	Beltsville

AM 1081-3, D77-5169, D77-4809, D77-5161, ARD-18, D77-18, 'Centennial', and 'Foster') were identified resistant and 1 line (AM 1081-1) was tolerant to SCN races 3 & 5. Additional lines PI 90763 and Foster were identified resistant while 'Peking' and AM 1974-5 were tolerant to both Pms races 2 & 11 (phytophthora root rot of soybean) and to Pseudomonas syringae pv. glycinea (bacterial blight of soybean) (Table 6).

One hundred and ten new improved soybean germplasm also have been screened for resistance to SCN races 3 & 5 in the field. The results indicated that 11 and 22 lines were identified resistant and tolerant, repectively (Table 7). Screening of the first 48 lines of the 110 improved soybean lines in the greenhouse indicated that 3 lines (AM X-1026 Pla 14-1, AM X-1026 Pla 14-3, and AM X-1065 Pla 15-1) were tolerant to SCN races 3 & 5. Forty-three lines initially were identifed resistant to Pms 2 & 11 (Table 8). Seven and 3 lines were resistant and tolerant, respectively, to Pseudomonas syringae pv. glycinea.

Improved soybean lines screened and selected for multiple resistance against SCN Races 3 & 5, Phytophthora root rot, and bacterial blight in the greenhouse Table 6.

Soybean cyst nematode	nematode	Phytophthora root rot	root rot	Bacterial blight	blight
Resistant lines	Tolerant lines	Resistant lines	Tolerant lines	Resistant lines	Tolerant
AM 1074-5	AM 1081-1	PI 90763	Peking	PI 90763	Peking
AM 1081-3	1	Foster	AM 1074-5	Foster	AM 1074-5
D77-5169					
D77-4809	Total - 1	Total - 2	Total - 2	Total - 2	Total - 2
D77-5161					

ARD 77 - 18

D77 - 18

Centennial

Foster

Table 7. Improved soybean lines screened and selected for resistance to soybean cyst nematode, Races 3 and 5

	— Soybean cyst nematode —————
Resistant lines	Tolerant lines
AMX-1026 P <sub>2a</sub> 14-2	AMX-1026 P <sub>la</sub> 14-1
AMX-1026 P <sub>3a</sub> 14-3	AMX-1026 P <sub>4a</sub> 14-4
AMX-1026 P <sub>9a</sub> 14-9	AMX-1026 P <sub>5a</sub> 14-5
AMX-1026 P <sub>15a</sub> 14-15	AMX-1026 P <sub>8a</sub> 14-8
AMX-1026 P <sub>18a</sub> 14-18	AMX-1026 P <sub>12a</sub> 14-12
AMX-1065 P <sub>27a</sub> 15-F	AMX-1026 P <sub>13a</sub> 14-13
AMS - 1002	AMX-1026 P <sub>16a</sub> 14-16
AMS - 1003	AMX-1065 P <sub>24a</sub> 15-4
AMS - 1004	AMX-1065 P <sub>26a</sub> 15-6
AM5549-1012-3-1	AMX-1065 P <sub>28a</sub> 15-8
P1040	AMX-1065 P <sub>29a</sub> 15-9
Total - 11	AMX-1065 P <sub>32a</sub> 15-12
	AMX-1066 P <sub>45a</sub> 16-7
	AMS - 1001
	AMS - 1002
	AMS - 1003
	AMS - 1013
	AM50 PL006-3
	AM51 PL 00 6-4
	AM53 PL 060
	AM54 Puniform 119
	$AM(49 \times 1069 - 2 - 1)$
	Total - 22

Improved soybean lines screened and selected for resistance to SCN Races 3 and 5 Phytophthora root rot, and bacterial blight in the greenhouse Table 8.

Soybean	Soybean cyst nematode	Phytophthora	root rot	Bacterial	1 blight
Resistant lines	Tolerant lines	Resistant lines	Tolerant lines	Resistant lines	Tolerant lines
None	AM X-1026 P <sub>1a</sub> 14-1 AM X-1026 P <sub>3a</sub> 14-3 AM X-1026 P <sub>21a</sub> 15-1 Total 3	AM X-1026 P <sub>1a</sub> 14-1 AM X-1026 P <sub>1a</sub> 14-2 AM X-1026 P <sub>1a</sub> 14-3 AM X-1026 P <sub>1a</sub> 14-4 AM X-1026 P <sub>1a</sub> 14-6 AM X-1026 P <sub>1a</sub> 14-6 AM X-1026 P <sub>1a</sub> 14-9 AM X-1026 P <sub>1a</sub> 14-9 AM X-1026 P <sub>1a</sub> 14-11 AM X-1026 P <sub>1a</sub> 14-12 AM X-1026 P <sub>1a</sub> 14-12 AM X-1026 P <sub>1a</sub> 14-12 AM X-1026 P <sub>1a</sub> 14-13 AM X-1026 P <sub>1a</sub> 14-15 AM X-1065 P <sub>21a</sub> 15-1 AM X-1065 P <sub>21a</sub> 15-1 AM X-1065 P <sub>21a</sub> 15-5 AM X-1065 P <sub>21a</sub> 15-5	None None 1 2 4 4 4 5 5 5 5 5 7 7 7 7 7 5 8 8 8 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	AM X-1026 P <sub>1a</sub> 14-1 AM X-1026 P <sub>1a</sub> 14-2 AM X-1026 P <sub>1a</sub> 14-4 AM X-1026 P <sub>1a</sub> 14-4 AM X-1066 P <sub>4</sub> 5 <sub>a</sub> 16-7 AM X-1066 P <sub>4</sub> 5 <sub>a</sub> 16-9 AM X-1066 P <sub>4</sub> 5 <sub>a</sub> 16-9 Total 7	AM X-1026 P <sub>6a</sub> 14-6 AM X-1066 P <sub>14</sub> 16-6 AM X-1065 P <sub>22a</sub> 15-2 Total 3

Table 8. Continued

Soybean cyst nematode	nematode	Phytophthora root rot	root rot	Bacterial bl	blight
Resistant	Tolerant	Resistant	Tolerant	Resistant	Tolerant
		P <sub>21a</sub>	70		
		AM X-1065 P <sub>21a</sub> 15-7	7		
		AM X-1065 P <sub>21a</sub> 15-8	80		
		AM X-1065 P <sub>21a</sub> 15-9	6		
		AM X-1065 P <sub>213</sub> 15-10	10		
		AM X-1065 P <sub>213</sub> 15-11	11		
		AM X-1065 P <sub>213</sub> 15-12	12		
		AM X-1065 P <sub>21a</sub> 15-13	13		
		AM X-1065 P <sub>21a</sub> 15-14	14		
		AM X-1065 P <sub>21a</sub> 15-15	15		
		AM X-1065 P <sub>21a</sub> 15-16	16		
		AM X-1065 P <sub>21a</sub> 15-17	17		
		AM X-1065 P <sub>213</sub> 15-18	18		
		AM X-1066 P <sub>393</sub> 16-1	1		
		AM X-1066 P <sub>39a</sub> 16-3	3		
		AM X-1066 P <sub>39a</sub> 16-4	\tau_1		
		AM X-1066 P <sub>39a</sub> 16-5	10		
		AM X-1066 P <sub>39a</sub> 16-6	٧٥		
		AM X-1066 P <sub>398</sub> 16-7	7		
		AM X-1066 P <sub>399</sub> 16-8	8		
		X-1066	6		
		AM X-1066 P <sub>39a</sub> 18-10	01		
		Total 43			

- II. To evaluate soybean germplasm for biological efficiency in:
  - a. Harvest Index
    (Dadson, R. B., J. Joshi, and L. Murphy, University of Maryland,
    Eastern Shore, Maryland)

Two hundred soybean plant introductions and cultivars in each of maturity groups III, IV, and V were evaluated during the 1983 planting season. Data collection consisted of days from emergence to maturity, final plant height, and oven-dried weights of stems and pod walls and seeds from four plants removed from each plot. Seed yield was determined by clipping off the end 30 cm of each plot and harvesting the remaining 1 m of the three rows. This report is limited to data on seed yield efficiency (SYE) values only.

A wide range of SYE was found in each maturity group (Tables 9, 10, and 11). SYE in MG III ranged from 0.43 to 1.39 with a mean of 0.88. About 60 entries had a higher mean SYE than 'Williams 79' which was used as a standard. In comparison, SYE in MG IV ranged between 0.44 and 1.27 with a mean of 0.83. Ninety-eight PIs in MG IV had SYEs higher than 'Clark' or 'Columbus', the standards. MG V had the widest range in SYE values from 0.25 to 1.56 and the highest mean, 0.97. Both 'Essex' and 'York', standard varieties on the eastern shore, had similarly high SYE, 1.22 and 1.21, respectively. Only 20 PIs had higher SYE than those two cultivars. Correlations between SYE, yield and plant height will be determined in each group and reported in the future.

b. Photosynthetic activity and translocation of photosynthates (Bhagsari, D. S., Fort Valley State College, Georgia)

Sixteen soybean genotypes comprising the parentage of cultivar Forrest were grown in pots in the greenhouse and under field conditions to determine whole-plant and single-leaf net photosynthesis (Pn), leaf-area index, dry-matter accumulation, photosynthate partitioning, leaf conductance, and yield. Under field conditions, the range in pN was 47.9 to 29.6 mg  $\rm CO_2dm^{-2}h^{-1}$  for 'Illini' and 'Haberlandt', respectively (Tables 12 and 15). Illini also had higher photosynthesis (whole-plant basis) than all other genotypes grown in pots (Table 13). The range in leaf area index was 5.8 to 3.4. 'Volstate' accumulated more dry matter (505.0 g/m²) than all the other genotypes during the vegetative stage of growth (Table 14). At pre-flowering stage, about 46.0% of the dry matter was partitioned to leaves (Table 15). Lower leaf surface showed more leaf conductance than upper leaf surface (1.0 and 0.30, respectively) in mid-September (night temperature 70°F). During the fall (night temperature 47°F), the leaf conductance was reduced to one third for both leaf surfaces on 23 September.

The yield data are not yet available.

Table 9. Ranking of seed yield efficiency (SYE) in soybean cultivars and plant introductions in Maturity Group III

	plant i	ntroductions i	n Matur	ity Group I	II		
Cultivar		Cultivar		Cultivar		Cultivar	
or PI #	SYE	or PI #	SYE	or PI #	SYE	or PI #	SYE
77 - 7. 1. 2 4	1 20	FC31.678	0.96	84.657	0.88	84.631	0.73
Hobbit	1.39	79.874	0.96	FC02.108	0.87	68.479-1	0.73
FC02.109	1.38		0.96	68.806	0.87	70.202	0.77
79.726	1.23	Ford		70.469-1	0.87	81.031-1	0.77
68.731	1.22	68.560	0.95	79.874-1	0.87	81.761A	0.77
79.587	1.19	68.756	0.95	I .		84.656	0.77
70.188	1.18	70.253	0.95	80.844-2	0.87	57.334	0.76
68.535	1.17	70.528	0.95	54.591	0.86	84.957-1	0.76
Fayette	1.14	81.037-3	0.95	54.618	0.86	70.541	
80.461	1.14	81.041-1	0.95	55.089-1	0.86	Ť	0.75
84.987	1.14	84.987-A	0.95	61.940	0.86	83.945-1	0.75
FC04.002B	1.13	65.379	0.94	68.692-2	0.86	84.581	0.75
68.701	1.12	70.466-4	0.94	68.761-3	0.86	84.644	0.75
70.566	1.12	71.461	0.94	84.646	0.86	80.844-3	0.74
69.993	1.10	79.583	0.94	68.479	0.85	81.766	0.74
54.613	1.09	68.533-2	0.93	70.023	0.85	84.979	0.74
79.620	1.09	71.845	0.93	79.848-1	0.85	79.835	0.73
54.608-5	1.08	72.232	0.93	84.957	0.85	81.044-1	0.73
69.515	1.08	85.009-2	0.93	62.483	0.84	82.246-1	0.73
79.628	1.08	Williams 79	0.92	70.469	0.84	84.682	0.73
84.976-1	1.08	FC29.333	0.92	70.501	0.84	84.908-2	0.72
79.645	1.07	FC31.572	0.92	79.691	0.84	FC19.979-2	0.60
79.710	1.07	54.610-1	0.92	79.693	0.84	54.620	0.69
82.302	1.07	60.272	0.92	82.308	0.84	60.296-2	0.69
68.732-1	1.05	68.748-1	0.92	84.579	0.84	80.459	0.69
70.213	1.05	70.247	0.92	68.523	0.83	81.031-2	0.68
70.519	1.05	70.473	0.92	68.599	0.83	85.019	0.68
68.533-1	1.04	84.976	0.92	81.030-1	0.83	84.611	0.67
70.019	1.07	68.423	0.91	84.757	0.83	84.662	0.67
70.500	1.03	70.212	0.91	54.620-2	0.82	84.914	0.67
79.627	1.02	68.470	0.90	68.398	0.82	68.621	0.65
84.509	1.02	68.494	0.90	70.001	0.82	68.648	0.65
79.797	1.02	68.528	0.90	70.462	0.82	81.667	0.64
79.872	1.02	69.995	0.90	70.199	0.81	80.480	0.63
68.710	1.02	70.080	0.90	81.044	0.81	81.780	0.63
70.014	1.02	70.470	0.90	83.940	į,	70.201	0.63
54.615		79.692	0.90	68.521-1	0.80	81.041	0.62
	1.01	80.825	0.90	70.192	0.80	82.235	0.62
54.615-1	0.99	80.841	0.90	80.831	0.80	85.292	0.60
79.616	0.99			80.481	0.80	84.619	0.59
79.691-4	0.99	84.666	0.90	84.610	0.80	80.845-1	0.59
70.076	0.98	68.609-1	0.88		- 1	62.202	
70.189	0.98	68.648	0.88	84.680	0.80		0.57
70.515	0.98	70.469-1	0.88	84.973	0.80	84.578	0.56
54.608-2	0.98	70.471	0.88	68.530-2	0.79	80.847-1	0.53
79.760	0.98	70.471	0.88	FC03.654N	0.78	80.845-2	0.52
79.870-2	0.98	71.850-1	0.88	54.583	0.78	82.232	0.46
FC31.571	0.97	80.822	0.88	68.759	0.78	81.037-2	0.43
FC31.684	0.97	81.038	0.88	80.471-1	0.78	Mean	.88
						S.D.	.16
						Variance	.03

Table 10. Ranking of seed yield efficiency (SYE) of soybean cultivars and plant introductions in Maturity Group IV

Cultivar or PI #	SYE	Cultivar or PI #	SYE	Cultivar or PI #	SYE
83.858	1.27	19.986	0.89	19.979-3	0.74
80.498-1	1.20	79.743	0.89	82.218	0.74
83.891	1.17	80.847-2	0.89	82.246	0.73
80.834-1	1.15	81.023	0.89	54.606-1	0.72
70.013	1.14	81.042-2	0.89	62.248	0.72
83.889	1.14	82-263.1	0.89	84.646-2	0.72
64.747	1.13	79.870-4	0.88	54.617	0.71
80.837	1.12	83.893	0.88	70.825-1	0.71
82.264	1.12	83.944	0.88	80.030	0.71
62.199	1.10	84.671	0.87	84.633	0.71
62.202-2	1.09	84.724	0.87	84.660	0.71
79.696	1.08	63.945	0.86	19.979-5	0.70
83.881	1.08	82.555	0.86	60.269-2	0.70
80.466-2	1.07	83.868	0.86	79.732-4	0.70
83.923	1.05	83.892	0.86	81.764	0.70
68.768	1.03	68.449	0.85	82.326	0.70
82.307	1.02	69.507-1	0.85	Clark	0.69
83.853	1.01	82.210	0.85	54.610-4	0.69
80.828-2	0.99	70.467	0.84	58.955	0.69
80.777	0.97	80.473	0.84	81.042-1	0.69
54.615-2	0.95	84.628	0.84	83.946	0.69
84.713	0.95	FC31.630	0.83	Columbus	0.68
54.600	0.94	54.614	0.83	70.243	0.68
70.229	0.94	56.563	0.83	81.037-5	0.68
70.242-2	0.94	70.208	0.83	82.325	0.68
82.558	0.94	82.259	0.83	FC31.946	0.67
79.732-3	0.93	19.979-1	0.82	59.849	0.67
82.312N	0.93	63.468	0.82	64.698	0.67
84.639	0.93	79.870.6	0.82	83.945-4	0.67
70.490	0.92	84.664	0.82	55.887	0.66
82.509	0.92	80.834-1	0.81	68.011	0.66
82.527	0.92	19.976-2	0.80	Douglas	0.61
80.479	0.91	71.444	0.80	19.979-7	0.61
80.828-1	0.91	83.881A	0.80	54.608-4	0.58
19.976-1	0.91	19.979-6	0.79	82.295	0.57
FC31.685	0.90	81.029-1	0.78	83.925	0.55
72.227	0.90	FC33.243	0.77	FC31-715	0.54
80.488	0.90	71.506	0.76	82.534	0.52
81.037	0.90	80.034-1	0.76	54.606-2	0.49
83.915	0.90	84.594	0.76	Cutler	0.46
84.679	0.90	84.669N	0.75	82.296	0.44
				Mean	0.83
				SD	0.16
				Variance	0.03

Table 11. Ranking of seed yield efficiency (SYE) of soybean cultivars and plant introductions in Maturity Group  ${\tt V}$ 

Cultivar		Cultivar		Cultivar		Cultivar	
or PI #	SYE	Cultivar or PI #	SYE	Cultivar or PI #	SYE	Cultivar or PI #	SYE
416.803	1.56	423.726	1.14	417.379	0.97	417.099	0.84
423.720	1.47	423.773	1.14	423.732	0.97	417.309	0.84
417.259	1.38	416.849	1.13	417.420	0.96	417.404	0.84
416.820	1.37	416.899	1.12	417.350	0.96	416.804	0.83
423.723	1.37	417.048	1.12	416.860	0.95	416.977	0.83
417.402	1.35	417.494	1.12	423.782	0.95	417.253	0.83
423.724	1.33	417.419	1.11	416.861	0.94	417.360	0.83
423.775	1.31	423.758	1.11	416.957	0.94	417.372	0.83
423.799C	1.31	423.764	1.09	417.031	0.94	417.026	0.81
417.332	1.29	416.799	1.08	417.373	0.94	417.068	0.81
417.423	1.29	416.844	1.08	417.430	0.94	417.073	0.81
423.742	1.29	417.284	1.07	416.808	0.93	423.727	0.81
417.392	1.28	417.308	1.07	923.759	0.93	416.909	0.80
417.263	1.27	417.390	1.07	416.843	0.92	417.159	0.79
423.762	1.26	417.486	1.07	416.960	0.92	417.383	0.78
417.037	1.25	423.785	1.07	416.979	0.92	417.157	0.76
423.751	1.25	416.797	1.06	417.069	0.92	417.158	0.76
417.581	1.24	417.090	1.06	417.074	0.92	417.474	0.76
416.877	1.23	417.341	1.06	423.745	0.92	416.827	0.74
417.440	1.23	417.411	1.06	417.055	0.91	417.346	0.74
Essex	1.22	416.982	1.05	417.414	0.91	417.329	0.72
417.493	1.22	417.280	1.05	416.847	0.90	416.927	0.70
417.247	1.22	417.098	1.04	416.901	0.90	417.275	0.70
York	1.21	423.774	1.04	417.081	0.90	416.815	0.68
416.962	1.21	416.821	1.03	417.264	0.90	416.931	0.68
417.251	1.21	417.352	1.03	423.781B	0.90	417.250	0.68
417.475	1.21	417.418	1.02	417.049	0.89	416.908	0.66
423.761	1.21	417.041	1.01	417.169	0.89	417.394	0.66
417.387	1.20	417.348	1.01	417.188	0.89	416.944	0.65
417.465	1.20	417.366	1.01	417.399	0.89	417.093	0.65
417.472	1.20	417.491	1.01	416.973	0.89	417.567	0.65
417.335	1.19	417.351	1.00	423.738	0.89	417.166	0.64
423.801	1.19	í	1.00	416.975	0.88	417.108	0.63
417.441	1.18	417.088	0.99 0.99	417.105	0.88	417.359	0.63
423.772	1.18	417.347	0.99	417.356	0.88	417.058	0.62
423.781A	1.17	417.415	0.99	417.483	0.88	417.00	0.61
416.970	1.17	417.445	0.99	416.807	0.87	417.053	0.58
417.464	1.17	416.851	0.98		0.87	417.396	0.58
417.467	1.16	417.039	0.98	417.272	0.87	417.052	0.56
416.999 417.481	1.15	417.106	0.98	416.811	0.86 0.86	417.135	0.56
423.76	1.15	417.141	0.98	417.016	0.86	417.322	0.52
416.800	1.14	417.426	0.98	423.786		423.804	0.44
416.800	1.14	417.492	0.98	416.981	0.86 0.86	417.337	0.42
416.838	1.14	423.722	0.98	417.262	0.86	417.307	0.37
416.871	1.14	417.103	0.97	417.288	0.86	417.402	0.25
417.363	1.14	417.273	0.97	416.85	0.84	Moon	0.07
TI. • 707	1.14	, , , , , , ,	0.5.	710.03	0.04	Mean S.D.	0.97
						Variance	0.21 0.05
						variance	0.05

Table 12. Net photosynthesis and specific leaf weight for field grown soybean genotypes (1983)

	Net phot	osynthesis	Specific leaf wt
Genotype	$mgCO_2 dm^{-2}hr^{-1}$	mgCO <sub>2</sub> /hr/g dry wt	mg/cm <sup>2</sup>
Illini	47.87	94.30	5.10
D49-2491	46.95	88.64	5.39
CNS	45.71	95.13	4.81
Peking	44.38	99.21	4.45
Bragg	44.38	94.14	4.71
Dyer	43.09	81.44	5.31
Palmetto	42.83	94.98	4.50
Hill	41.00	79.01	5.23
Volstate	40.86	85.06	4.78
Dunfield	40.39	72.21	5.60
Jackson	39.45	83.54	4.74
Lee	36.88	81.07	4.73
S-100	36.29	82.99	4.52
Forrest	34.73	71.97	4.82
Clemson	31.27	66.55	4.62
Haberlandt	29.57	74.51	3.98
Mean	40.35	84.05	4.83

Table 13. Leaf area index for soybean (1983)

Genotype	LAI
S-100	5.81
Haberlandt	5.73
CNS	5.69
Volstate	5.64
Jackson	5.17
Palmetto	4.72
Bragg	4.61
Peking	4.48
Lee	4.44
Clemson	4.25
Dyer	4.04
Hill	4.02
Forrest	4.00
Dunfield	3.90
D49-2491	3.83
Illini	3.42
Mean	4.58

Table 14. Dry matter accumulation and partitioning for soybean genotypes 1983

Genotype	Dry matter g/m²	% in leaves
Volstate	505.0	43.8
Haberlandt	446.0	44.8
S-100	427.3	44.8
CNS	380.5	47.3
Jackson	376.2	42.5
Palmetto	364.4	43.8
Lee	346.6	47.5
Bragg	346.6	47.5
Dunfield	342.3	49.3
Hill	328.2	42.8
Dyer	309.9	48.8
Clemson	308.5	46.0
D49-2491	304.1	46.5
Illini	287.4	45.5
Peking	268.1	49.0
Forrest	251.7	45.8
Mean	349.5	46.0

Table 15. Canopy photosynthesis for soybeans grown in pots (1983)

	Photosy	nthetic rate (mgCO <sub>2</sub> /hı	-)
Genotype	per dm² leaf area	per g fresh wt	per g dry wt
Illini	16.56	11.52	35.46
Bragg	16.45	11.98	39.86
Dunfield	13.84	10.80	33.35
S-100	13.41	10.20	32.01
CNS	11.86	9.64	35.94
Peking	11.78	9.21	31.59
D49-2941	11.71	8.87	29.93
Lee	11.24	8.84	28.76
Hill	10.61	8.26	27.76
Haberlandt	10.52	7.17	24.62
Forrest	10.50	8.20	27.26
Jackson	10.16	9.43	32.77
Clemson	10.08	7.22	27.40
Dyer	8.68	6.83	22.25
Volstate	8.45	6.63	22.10
Palmetto	7.70	5.88	23.20
Mean	11.47	8.79	29.64

### C. Nitrogen Fixation (Sapra, V. T., Floyd, M., R. Garner and S. Mookherji, Alabama A&M University, Normal, AL)

Twenty commercial soybean cultivars from maturity groups IV through VIII were screened for nitrogen-fixation in a growth chamber using *Rhizobium* strains 3I1B 6,122 and combination of 6 and 122. The data on nodule number, nodule weight, shoot fresh weight, shoot dry weight, and acetylene reduction were recorded. Among twenty cultivars, 'Lee 74' (MG IV), 'Bay' (MG V), and 'Essex' (MG V) were identified as high N-fixers, based on more nodules and high acetylene reduction. The lower N-fixers showed low value of acetylene reduction for strains 6 and 122, as well as for 6+122 (Table 16). Several advance breeding germplasm lines are being screened in our laboratory for compatibility with these strains.

Table 16. Response of two rhizobia strains and their combination on nodule number in different maturity groups of soybean cultivars

Maturity Group		Rhizobium strain	
and cultivar	6	122	6+122
MG IV			
RA 480	8.3 c <sup>a</sup>	6.9 feg	7.0 dc
RA 401	11.0 cd	10.9 fbdecg	9.8 bdc
Stevens	5.0 c	5.4 g	8.3 bdc
MG V			
Bedford	6.3 c	9.4 fdecg	7.8 dc
Forrest	9.7 c	11.4 fbdecg	7.7 dc
Wilstar 550	10.6 cb	9.9 fdecg	7.1 dc
Essex	17.1 Ъ	15.2 bac	18.2 a
Bay	24.8 a	20.7 a	18.7 a
MG VI			
Lee 74	17.1 Ъ	17.1 ba	14.6 ba
Tracy	12.4 cd	14.8 bdac	11.9 bdc
Davis	10.5 cb	13.0 bdac	12.9 bac
Centennial	17.2 b	15.9 bac	14.4 ba
McNair 600	7.1 c	6.1 fg	7.2 dc
RA 680	10.8 cb	12.6 fbdec	7.7 dc
Greenseed 737	12.3 cb	9.9 fdecg	11.9 bdc
MG VII			
Bragg	5.1 c	7.6 feg	6.0 d
Braxton	5.5 c	7.7 feg	6.3 dc
Hutton	8.7 đ	8.6 fdeg	7.0 dc
MG VIII			
Foster	9.4	11.2 fbdecg	10.5 bdc
Wright	6.8 c	9.3 fdecg	7.8 dc

<sup>&</sup>lt;sup>a</sup>Means within a column with the same letter are not significantly different.

d. Micronutrient Uptake (Reddy, M. R., North Carolina Agricultural and Technical University, N.C.)

An investigation was carried out to evaluate soybean germplasm response to (1) manganese deficiency and toxicity, (2) various rates of manganese application, and (3) to different soil pH levels on manganese accumulation by the germplasm lines and their yield. Manganese rates were 0, 10, and 20 kg/ha. The pH levels were 5.3, 6.3, and 7.0. The germplasm lines tested were PI 159319, PI 324924, PI 960895 and L-76-0132. Results indicated differences in seed yield among the germplasm lines tested due to manganese rates and pH level (Table 17). Germplasm lines PI 960895 gave the highest seed yield; L-76-0132 produced lowest yield. Seed yields for germplasm lines PI 159319 and PI 960895 were higher at 20 kg/ha manganese application compared with control. In general, germplasm growth and yield were higher at pH 7 and 6.3 and poor at pH 5.3. Germplasm line PI 324924 showed normal plant growth and higher yield at pH 5.3. It appears that strongly acid condition of the soil at pH 5.3 resulted in manganese toxicity to the germplasm lines except for line PI 324924. The data suggest differences in tolerance to toxicity of Mn by various germplasm lines under strong acid condition of soil.

Table 17. Effect of rate of manganese and soil pH levels on seed yield of soybean germplasm

рН	PI 159,319	PI 324,924	PI 960,895	L-76-0132
levels		g/p1a	ant	
		Mn: OK	kg/ha	
7.0	12.75	9.71	10.31	3.47
6.3	13.92	11.12	15.10	5.49
5.3	3.85	15.57	13.79	3.75
	30.51	36.40	30.20	12.71
		Mn: 10	Kg/ha	
7.0	8.38	5.20	10.91	5.43
6.3	9.31	7.35	10.32	6.49
5.3	5.09	9.03	9.97	5.70
	22.78	21.58	31.20	17.62
		Mn: 20	Kg/ha	
7.0	18.60	7.00	13.57	3.83
6.3	11.91	7.61	10.97	4.47
5.3	10.54	11.50	12.21	3.93
	41.05	26.11	36.75	12.23
	94.35	84.09	98.15	42.56

#### (J. R. Allen, Tuskegee Institute, AL)

Two cultivars recommended for central Alabama, 'Bragg' and 'Lee', were selected for the screening process. The two cultivars were planted in plots 4 x 6m in which Mn or Zn were added at the rates of 0, 4, 8, and 16 kg/ha. There were three rows per plot and the soil was a Norfolk sandy loam (fine, loamy siliceous, thermic typic paleudult). Each micronutrient was added to plots by broadcasting, then lightly disced into the soil. After micronutrients were added, Rhizobium-inoculated seed of each soybean cultivar were planted in each row at the rate of 45 kg/ha. At the blooming stage, five plants from each plot were harvested for Mn and Zn determination. The remaining plants were allowed to remain until pod-ripening stage and then harvested for total dry matter and seed yield determination.

Tissue analyses for Mn and Zn concentration are not yet available; however, dry-matter and seed-yield analysis showed that both Bragg and Lee are directly affected by soil Mn and Zn concentration. Seed yield of Lee in the Mn-treated plots tended to be lower than that of Bragg. In addition, at all levels of applied Mn, seed yield of Lee was lower than the control (Table 19). For the Zn-treated plots, seed yield of Bragg at all levels at pH 6.5 varied little from the control. Conversely, seed yield of Lee for the same pH level was generally lower for the 8-to-16-kg/ha rates than control plants. While seed yield of Bragg was considerably higher at pH 6.5 than at pH 5.0, that of Lee, although higher at pH 6.5 than at 5.0, the difference in yield between the two pH levels was not as great as it was for Bragg. At pH 6.5, total dry-matter accumulation was highest with the 4 kg/ha of both Mn and Zn. Increasing the concentration of these two micronutrients above this level tended to decrease dry-matter production. Generally, at pH 5.0, dry-matter production tended to be lower than control plants at all levels of applied Zn (Table 18).

The effect of Mn and Zn on seed yield was somewhat more variable than for total dry matter. Generally, however, seed yield of Bragg at pH 6.5 in the Mn-treated plots was slightly higher for the 8-to-16 kg/ha rates than the control plants (Table 19).

Table 18. Effect of Mn and Zn on total dry matter yield of Bragg and Lee

Micronutrient		M	ĺn	Zn	
concentration	рН	Bragg	Lee	Bragg	Lee
0 Control	5.0	12.6	9.8	22.9	11.7
	6.0	13.7	8.5	19.0	12.9
4	5.0	10.1	7.9	15.5	19.1
	6.5	25.2	12.6	26.2	19.4
8	5.0	11.8	12.0	12.9	11.9
	6.5	17.0	16.9	20.6	10.2
16	5.0	14.1	10.8	8.6	12.9
	6.5	12.6	15.9	4.3	17.0

Table 19. Effect of Mn and Zn on seed yield of Bragg and Lee

Micronutrient		M	n	Zn		
concentration	рН	Bragg	Lee	Bragg	Lee	
			kg/l	na ————		
0 Control	5.0	1887	1847	1733	2676	
	6.5	1973	2472	5468	3060	
4	5.0	1994	1609	2360	2734	
	6.5	1715	1689	5417	2830	
8	5.0	1693	2213	2238	2299	
	6.5	2121	1445	4205	3089	
16	5.0	1506	1575	2866	3692	
	6.5	2317	2206	5102	2913	

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# 1) In vitro fertilization of perennial soybean species.

Interspecific hybridization in the genus *Glycine* is difficult and few successes have resulted from a multitude of attempts (Newell and Hymowitz, 1982, 1983). Because flowers of the perennial species are especially difficult to manipulate in the field or greenhouse, a study was undertaken to examine the potential of fertilizing these species *in vitro*, thereby partially alleviating mechanical damage to the ovulary and environmental stress. Initial studies were intended to develop *in vitro* procedures under which ovularies would survive and form viable embryos. Therefore, all flowers were self-fertilized.

Seeds of perennial species *G. clandestina*, *G. falcata*, *G. latifolia*, and *G. tabacina* were obtained from Dr. R. L. Bernard and planted in the field and greenhouse. Flowers from greenhouse plants were used exclusively as explant material because those from field plots could not be disinfested adequately. Flowers were collected immediately before dehiscence and disinfested with 33% commercial clorox solution. The corolla and androecium were removed. Pollen was applied to the stigma from a more mature flower of the same plant and the pistil with intact calyx implanted on agar-based medium in a 10 mm glass vial.

The most successful medium for pistil development consisted of an equal volume mixture of Murashige-Skoog and Gamborg B-5 mg salt formulations supplemented with 0.05 mg BA (benzyladenine), 0.5 mg  ${\rm GA}_3$  (gibberellin), 2.0 mg thiamine-HCl, 0.5 mg pyridoxine-HCl, 250 mg myo-inositol, 100 mg casein hydrolysate and 5.5 g Difco Bacto-agar per liter of solution. Sugar concentrations of 3% and 5% were more supportive of pod and embryo development than 10% (Table 1). Greater concentrations of the cytokinin BA caused extensive callus formation at the base of the pistil.

Embryos that matured to the stage at which they could be easily excised, usually after about four weeks, were subcultured on the medium described by Cutter and Bingham (1975) where shoots and roots developed. The plants were then easily transferred to potting mix.

Viable embryos were recovered from all species (Table 1). All but  ${\it G}$ . falcata were successfully cultivated into mature, fertile plants. Although the recovery percentage was not high, averaging only 9% overall, we expect improvement with increased experience.

Additional *in vitro* studies may be useful in facilitating wide hybridizations, thus expanding the soybean germplasm base.

Influence of sucrose concentration on the incidence of pod and embryo development in vitro\* Table 1.

		Sucr	ose concentrat	Sucrose concentration (grams/liter)	er)	1
	30	0	50		1(	100
Species	Pistils implanted	Embryos recovered	Pistils implanted	Embryos recovered	Pistils implanted	Embryos
G. clandestina	30	7	17	√	28	1
G. falcata	9	0	1	<b>¦</b>	80	E
G. latifolia	1	1	07	7	28	æ
G. tabacina	100	10	14	p	38	R
All species	136	1.7	71	10	204	10
Percent recovery		12.5		14.1		6.4

\*No pod contained more than one embryo.

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1) Instability of virulence characters of soybean mosaic virus strains after seed transmission//

Soybean mosaic virus (SMV) is distributed worldwide. The primary mode of transmission is through seed (Goodman and Oard, 1980), although the virus is also nonpersistently transmitted by aphids (Irwin and Goodman, 1981). Cultivars have been identified which combine tolerance to SMV infection and low incidence of transmission through seed (Goodman and Oard, 1980).

Several reports exist of the variety in symptoms caused by various isolates of SMV observed in various soybean cultivars. In 1979, Cho and Goodman classified 98 isolates of SMV from seeds in the USDA soybean germplasm collection into seven strains (Gl - G7) based on disease reactions of soybean cultivars to these strains. The authors recommended that a range of SMV strains differing in virulence should be used in breeding programs in which SMV resistance is an objective. A few years later, Polston, Xu, and Goodman (unpublished) identified several additional strains of SMV from the People's Republic of China. These were more virulent than Gl - G7, and produced different reaction patterns on the differential soybean lines than did the seven classified strains.

Recent evidence suggests that even the seven characterized strains (G1 - G7) may possess unstable virulence characteristics when they are transmitted through seeds. Virus isolates recovered from seedlings of parents inoculated with a particular strain may not match the differential host reactions of the inoculated strain. Implications of this instability may be significant in breeding programs.

Materials and methods: Two strains of SMV, G2 and G4, were obtained from infected tissue dried and stored over silica gel or calcium sulfate at -20°C by Cho after his classification studies. These were inoculated onto soybean cultivar 'Williams' for increase. The soybean cultivar 'Midwest' was used for transmission experiments, since it is reported to transmit SMV through seed at a rate of 21.7% (Bowers, 1980). Midwest seedlings were used 24 days after planting, approximately two weeks prior to flowering, at the 3-trifoliolate stage. All seedlings showing symptoms of virus infection were removed. One primary leaf from each remaining plant was frozen for later analysis by enzyme-linked immunosorbent assay (ELISA) to insure that all test plants were initially virus-free. For inoculations, SMV-infected leaf tissue was ground in 0.01 M sodium phosphate, pH 7.2, and 600-mesh carborundum was added to the slurry. Inoculum was rubbed onto test-plant leaves with a cotton-tipped applicator. Leaves were rinsed with tap water. Twenty plants were inoculated with G2, 20 with G4, and 20 received treatment with G2 and G4 (G2 was inoculated onto the youngest trifoliolate, G4 onto the next youngest). Test plants were kept in a greenhouse with supplemental fluorescent lights at 70-80°F until they flowered, set seeds, and reached seed maturity, about 11 weeks after inoculation. Shortly before test plants dried, leaves were collected from four plants of the G2 group, four of the G4 group, four of the group with G2 and G4, and three of the buffer controls. These samples were used to inoculate the soybean differential lines used by Cho and Goodman (1979) to confirm

the identity of the virus strains. Seeds from test plants were collected and dried at room temperature for two weeks, treated with Captan, and planted in sand benches in the greenhouse. Five days later, one primary leaf from each plant was collected for testing by ELISA. Four weeks after planting, young tissue was collected from each of the seedlings shown by ELISA to be infected with SMV. This tissue was used to inoculate sets of differential soybean lines to identify the strain(s) of SMV in the seedlings.

Results and discussion: The results of these experiments are summarized in Table 1. The identity of the SMV strain recovered from inoculated test plants at the end of the growth period was, in all cases, the same as that with which the plants were inoculated at the beginning of the experiment. Some of the seeds produced by these plants contained SMV, through seed transmission. However, the identity of the virus strain in the offspring seedling was not always the same as that present in the parent plant. We also found that Midwest plants which arose from seeds in our seed lot previously infected with SMV also produced seeds carrying a different SMV strain. It was not always possible to pinpoint the identity of a strain absolutely. For example, according to Cho and Goodman's (1979) scheme, if a sample causes mosaic symptoms in Williams and necrosis in 'Ogden' and 'Marshall', it could be strain G3 alone or a combination of strains G2 and G3 (Table 2). Nonetheless, our results indicate that the virulence of SMV transmitted through soybean seeds may be altered. The mechanism by which this apparent selection for altered virulence via seed transmission occurs is unknown.

Table 1. Identification of strains of SMV from soybean cultivar Midwest before and after transmission through seeds

	Strains of		
Used to inoculate virus-free parent plants		Identified in parent plants at	Identified in seed- lings from seeds of parent plant
G2		G2	G3 alone, or G2 and G3
G4		G4	G2 and G3 and G4
G2 and G4		G2 and G4	G3 alone, or G2 and G3
Buffer		No virus	No virus
	G2	Not done	G3 alone, or G2 and G3

<sup>&</sup>lt;sup>a</sup>Identification of SMV strains was done by inoculation onto differential soybean lines (Cho and Goodman, 1979).

Table 2. Reactions of SMV strains G2, G3 and G4 on differential soybean lines of Cho and Goodman (1979)

Virus			— Soybean	cultivar —		
strain	Williams	York	Ogden	Marshall	Kwangyo	Buffalo
G2	м <sup>а</sup>			N		
G3	М		N	N		
G4	M	M/N				

<sup>&</sup>lt;sup>a</sup>Symbols for symptoms: -- = symptomless, no virus detected by assay on bean; M = mosaic; N = necrosis.

Acknowledgements: We would like to thank Susan E. Rheingans and Janet A. Leath for excellent technical assistance, and Jane E. Polston for helpful discussion and advice.

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# 1) The genus Glycine subgenus Glycine germplasm collection

The genus *Glycine* as currently delimited is divided into two subgenera *Glycine* and *Soja*. The subgenus *Soja* includes the soybean, *G. max*, and its annual wild counterpart, *G. soja*. The subgenus *Glycine* comprises seven wild perennial species. The collection of wild perennial *Glycine* species held at the University of Illinois stands at 288 accessions; a program of morphological, biochemical, and cytogenetic analysis currently is in progress. Voucher specimens of all accessions are deposited in the herbarium of the Crop Evolution Laboratory (CEL), University of Illinois at Urbana-Champaign.

Species	No. of accessions	<u>2n</u>	Country
G. canescens	35(27) <sup>a</sup>	40	$A^{b}(NT,V,NSW,SA,WA)^{c}$
G. clandestina	54(49)	40	A(ACT, NSW, Q, SA, V, T)
G. falcata	4(3)	40	A(Q)
G. latifolia	10(10)	40	A(Q,NSW,UN)
G. latrobeana	6(1)	40	A(V)
G. tabacina	87(64)	40,80	A(NSW,A,ACT),TA,UN
			RI,F,NC,TO,VA,MI
G. tomentella	88(44)	38,40,78,80	A(NSW,NT,Q),TA,P,PNG
Unknown	4		

<sup>&</sup>lt;sup>a</sup>Number of accessions (cytology completed).

T. (Hymowitz R. J./Singh

# 2) Hybridization in genus Glycine subgenus Glycine//

Since the publication of Newell and Hymowitz (1983), we have obtained a considerable number of new intra- and interspecific hybrids in subgenus *Gly-cine*. Parents in the hybridization programs were selected based upon their differences in morphology, chromosome number, and the area of origin. These hybrids shown below are being studied cytogenetically.

bCountry abbreviations: A = Australia, F = Fiji, MI = Mariana Islands, NC = New Caledonia, P = Philippines, PNG = Papua New Guinea, RI = Ryukyu Islands, TA = Taiwan, TO = Tonga, UN = Unknown, and VA = Vanuatu.

C(Regions within Australia): ACT = Australian Capital Territory; NSW = New South Wales; NT = Northern Territory; Q = Queensland; SA = South Australia, T = Tasmania; UN = Unknown; V = Victoria; and WA = Western Australia.

Table 1. Intraspecific hybrids

	Origin	0	0	NSW	NC	NSW	MI	TO	ò	NSW	NSW	RI	NC	TA	TA	NSW	0
Male	2n	07	07	80	80	80	80	80	80	80	80	80	80	80	80	78	80
	Species	CLA	LAT	TAB	TOM	TOM	TOM	TOM									
	Origin	MSN	S	NC	MSN	MI	MSM	MSN	ACT	RI	TO	TO	VA	ò	0	0	0
Female	$\frac{2n}{}$	07	70	80	80	80	80	80	80	80	80	80	80	78	80	80	40
	Species	CLA	LAT	TAB	TOM	TOM	TOM	TOM									
	Origin	MSN	SA	MSN	MI	RI	ACT	RI	VA	NSW	VA	NC	NSM	Ò	ò	Ò	NSM
Male	2 <u>n</u>	07	70	70	80	80	80	80	80	80	80	80	80	80	78	78	78
	Species	CAN	CAN	TAB	TOM	TOM	TOM	TOM									
	Origin <sup>b</sup>	SA	MSN	NSW	RI	MI	MSN	NSM	MSN	ACT	RI	TO	VA	0	TA	PNG	TA
Female	2n	40	70	07	80	80	80	80	80	80	80	80	80	80	80	40	80
174	Species	CANa	CAN	TAB	TOM	TOM	HOL	TOM									

Table 2. Interspecific hybrids

Female         Male         Female         Female         Male	A. Two-v	Two-way hybrids	rids									
2n         Origin         Species         2n         Origin         Species         2n         Origin         Species         2n           40         NSW         TOM         AG         TOM         TOM         80           40         AGT         LAT         40         NSW         TAB         40           40         NSW         CLA         40         AGT         TOM         80           40         VN         TAB         40         NSW         CLA         40         AG           40         VN         TAB         40         NSW         LAT         40         NSW         CLA         40           40         VN         TAB         40         NSW         LAT         40         NSW         TAB         80           40         VN         TOM         80         TA         TOM         70         80           40         VN         TOM         NSW         TAB         NSW         TAB         80         AG           40         VN         TOM         NSW         TAB         NSW         TAB         AG         AG         AG           40         VN         NSW	H.	emale			Male			Female			Male	
40         NSW         TOM         78         NSW         CAN         40         NSW         TOM         80           40         ACT         LAT         40         UN         CLA         40         ACT         TAB         40           40         NSW         TAB         SA         LAT         40         UN         CAN         40           40         UN         CAN         40         W         LAT         40         UN         40           40         UN         TOM         40         NSW         LAT         40         UN         TOM         80           40         UN         TOM         40         NSW         TAB         80         40           40         UN         TOM         NSW         TAB         80         7         CAN         40           40         UN         TOM         NSW         TAB         80         7         CAN         40           40         WSW         TOM         W         TOM         40         W         40         A         40         A           80         Q         CAN         40         W         A         A	Species	2n	Origin	Species	2n	Origin	Species	2n	Origin	Species	2 <u>n</u>	Origin
40         ACT         LAT         40         UN         CLA         40         ACT         TAB         40           40         NSW         TAB         80         NSW         CLA         40         ACT         TOM         80           40         UN         CAN         40         Q         LAT         40         UN         CAA         40           40         UN         TAB         40         NSW         LAT         40         UN         TAB         80           40         UN         TOM         80         TA         TOM         80         TA         A0         V         TOM         80           40         NSW         CAN         40         NS         TAB         80         T         A0	CAN	07	NSM	TOM	78	MSN	CAN	40	NSM	TOM	80	0
40         NSW         TAB         80         NSW         CLA         40         ACT         TOM         ACT         TOM         80           40         UN         CAN         40         CAN         LAT         40         UN         CAA         40           40         UN         TAB         40         NSW         LAT         40         UN         TAB         80           40         UN         TOM         80         TA         AO         UN         TOM         80           40         UN         TOM         40         NSW         TAB         80         T         AO           40         WSW         TAB         NSW         TAB         80         T         AO         AO           40         WSW         TAB         NSW         TAB         AO         AO         AO         AO           40         Q         CAN         40         AO         AO         AO         AO         AO         AO           80         Q         AO         AO         AO         AO         AO         AO         AO         AO           80         Q         AO         AO	CLA	07	ACT	LAT	40	N	CLA	07	ACT	TAB	07	NSM
40         UN         CAN         40         CAN         40         UN         40         UN         CAN         40           40         Q         CLA         40         Q         LAT         40         NSW         CLA         40           40         UN         TOM         NSW         LAT         40         UN         TOM         80           40         UN         TOM         80         TA         40         UN         TOM         80           40         NSW         CAN         40         W         TOM         40         40           40         NSW         TAB         80         T         CAN         40           40         NSW         TAB         80         T         CAN         40           40         Q         CAN         40         Q         T         CAN         40           80         Q         CAN         40         Q         Q         CAN         40	CLA	07	NSM	TAB	80	MSM	CLA	40	ACT	TOM	80	ò
40         Q         CLA         40         Q         LAT         40         NSW         LAT         40         NSW         TAB         60         W         TAB         80           40         UN         TOM         PNG         LAT         40         UN         TOM         80           40         UN         TOM         80         TA         TAB         80         TOM         80           40         NSW         CAN         40         NSW         TOM         40         Q         40           80         Q         CAN         40         NS         TOM         40         Q         40           80         Q         CAN         40         NS         TOM         40         40	LAT	07	N	CAN	40	SA	LAT	70	N	CAN	07	MSN
40         UN         TAB         40         NSW         LAT         40         UN         TAB         80           40         UN         TOM         PNG         LAT         40         UN         TOM         80           40         UN         TOM         NSW         TAB         80         T         CAN         40           40         Q         CAN         40         V         TOM         40         Q         CLA         40           80         Q         CAN         40         SA         TOM         40         CLA         40	LAT	40	0	CLA	40	ò	LAT	40	MSN	CLA	07	NSM
40         UN         TOM         40         PNG         LAT         40         UN         TOM         80           40         UN         TOM         40         NSW         TAB         80         T         CAN         40           40         Q         CAN         40         V         TOM         40         Q         CLA         40           80         Q         CAN         40         SA         TOM         40         Q         CLA         40	LAT	70	N	TAB	40	MSN	LAT	40	N	TAB	80	NC
40         UN         TOM         80         TAB         80         T         CAN         40           40         NSW         CAN         40         V         TOM         40         Q         CLA         40           80         Q         CAN         40         SA         TOM         40         Q         CLA         40	LAT	07	NIO	TOM	40	PNG	LAT	40	N.	TOM	80	ò
40         NSW         40         NSW         TAB         80         T         CAN         40           40         Q         CAN         40         V         TOM         40         Q         CLA         40           80         Q         CAN         40         SA         CAN         40         Q         CLA         40	LAT	07	Nn	TOM	80	TA						
40         Q         CAN         40         V         TOM         40         Q         CLA         40           80         Q         CAN         40         SA         40 </td <td>TAB</td> <td>07</td> <td>NSW</td> <td>CAN</td> <td>07</td> <td>MSM</td> <td>TAB</td> <td>80</td> <td>H</td> <td>CAN</td> <td>0 7</td> <td>NSM</td>	TAB	07	NSW	CAN	07	MSM	TAB	80	H	CAN	0 7	NSM
80 Q CAN 40	TOM	07	O	CAN	40	Λ	TOM	70	ò	CLA	40	MSN
	TOM	80	ò	CAN	40	SA						

Continued . . .

Table 2. Continued

# 3. Three-way hybrids

• [CAN 
$$(2n = 40)$$
 NSW] x [TAB  $(2n = 80)$  NC x TAB  $(2n = 80)$  NSW]

[CLA 
$$(2n = 40)$$
 NSW x CAN  $(2n = 40)$  NSW] x [LAT  $(2n = 40)$  Q]

[LAT 
$$(2n = 40)$$
 NSW x CLA  $(2n = 40)$  Q] F<sub>1</sub>  $\stackrel{\text{GT}}{=}$  2n = 80 x [TOM  $(2n = 78)$  N

[LAT 
$$(2n = 40)$$
 NSW x CLA  $(2n = 40)$  Q] F<sub>1</sub>  $\stackrel{\text{GT}}{=}$   $2n = 80$  x [TAB  $(2n = 80)$  NSW]  
[LAT  $(2n = 40)$  NSW x CLA  $(2n = 40)$  Q] F<sub>1</sub>  $\stackrel{\text{GT}}{=}$   $2n = 80$  x [TAB  $(2n = 80)$  TA]

$$(2\underline{n} = 40)$$
 NSW x CLA  $(2\underline{n} = 40)$  Q] F<sub>1</sub>  $\stackrel{\subseteq T}{=}$   $2\underline{n} = 80$  x [TAB  $(2\underline{n} = 80)$  TA]  $(2\underline{n} = 40)$  NSW x CLA  $(2\underline{n} = 40)$  Q] F<sub>1</sub>  $\stackrel{\subseteq T}{=}$   $2\underline{n} = 80$  x [TAB  $(2\underline{n} = 80)$  VA]

$$(2\underline{n} = 40) \text{ NSW} \times \text{CLA} (2\underline{n} = 40) \text{ Q}] F_1 = 2\underline{n} = 80 \times [\text{TAB} (2\underline{n} = 80) \text{ NC}]$$

# C. Four-way hybrids

[LAT

[LAT

4.

[LAT 
$$(2n = 40)$$
 NSW x CLA  $(2n = 40)$  Q]  $F_1 \stackrel{\text{GT}}{=} 2n = 80$  x [TAB  $(2n = 80)$  NC x TAB  $(2n = 80)$  NSW]

<sup>a</sup>Species abbreviations: CAN = canescens, CLA = clandestina, LAT = latifolia, TAB = tabacina, and TOM = tomentella.

 $^{\mathrm{b}}$  For country or region code, see previous article.

# Reference

Hybridization in the genus Glycine subgenus Glycine Willd. (Leguminosae, Papilionoideae). Am. J. Bot. 70:334-348. 1983. Newell, C. A. and T. Hymowitz.

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# 3) A soybean x Glycine tomentella hybrid: Progress and problems

Newell and Hymowitz (1982) reported successful hybridization between the soybean (cv 'Altona') ( $2\underline{n}$  = 40) and a wild perennial relative *Glycine tomentella* (IL 428) ( $2\underline{n}$  = 78). The  $F_1$  plant was completely sterile ( $2\underline{n}$  = 3 $\underline{x}$  = 59). A graft of the  $F_1$  plant was made on to the cv 'Williams'. The chromosome number of the grafted plant was doubled ( $2\underline{n}$  = 6 $\underline{x}$  = 118) by treatment with 0.1% colchicine. The  $F_1$  colchicine-treated plant produced two pods containing three seeds. One seed germinated to produce an  $F_2$  plant ( $2\underline{n}$  = 6 $\underline{x}$  = 118). In 1983, we attempted to backcross several soybean cultivars to this plant. The results are shown below.

Female	Male No.	of flowers	Seed set
$F_2$ Altona x IL 428 (2 $\underline{n}$ =118)	Altona	131	0
$F_2$ Altona x IL 428 (2 $\underline{n}$ =118)	Clark 63	86	0
$F_2$ Altona x IL 428 (2 $\underline{n}$ =118)	Essex	425	0
$F_2$ Altona x IL 428 (2 $\underline{n}$ =118)	Williams	186	0
F <sub>2</sub> Altona x IL 428 (2 <u>n</u> =118)	Wye	170	0
		998	0

We were not successful in obtaining backcross progeny. However, the  $\rm F_2$  plant produced two pods containing two seeds each. The  $\rm F_3$  seeds will be germinated in April, 1984, and during the summer of 1984 attempts will be made to backcross soybean cultivars to the  $\rm F_3$  plant.

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# 1) Superoxide dismutase (SOD) isoenzymes in soybean,

We are using vertical polyacrylamide gel electrophoresis (Davis, 1964) and a staining system modified after Beauchamp and Fridovich (1971) to study superoxide dismutase polymorphisms in the subgenus soja. This staining system generates superoxide radical; hence, it is specific for SOD activity. We resolve up to 9 SOD bands in dry or germinating soybean cotyledons, and in leaves. Three zymogram patterns were observed.

- 1) All 9 bands present (cv. 'Century');
- 2) Bands 4 and 5 absent (cv. 'Evans');
- 3) Bands 8 and 9 with a slower migration rate (cv. 'Polysoy').

Based on cyanide-inhibition studies, bands 4 through 9 are the copperzinc form of the enzyme, which has been shown to be a dimer in all species studied (Fridovich, 1975; Baum and Scandalios, 1981). The zymogram patterns we observe for bands 4-6 and 7-9 are consistent with the model of two sets of dimeric, Cu-Zn isozymes which form interlocus heterodimers. Bands 1-3 are not inhibited by CN; we believe these to be the manganese enzyme, which has been shown to be a tetramer occurring in the mitochondria of all species studied to date (Fridovich, 1975; Baum and Scandalios, 1981).

The observed zymograms correspond closely to the int-oxidase patterns observed on a similar electrophoretic system (Larsen and Benson, 1970). There are also certain similarities to the tetrazolium oxidase zymograms described by Gorman and Kiang (1977). Based on a survey of over 150 soybean cultivars, we conclude that our SOD pattern 1 corresponds to the type-1 TO zymogram described by Gorman and Kiang; likewise, our SOD patterns 2 and 3 are equivalent to their TO zymogram types 2 and 3. Further, under these staining conditions, the  $\it Ep$  locus (for seed coat peroxidase) does not produce an achromatic band (band 4 of Gorman et al.).

Previous reports (Gorman and Kiang, 1978) indicated that the type 1 vs. type 2 TO zymograms were conditioned by a single locus,  $T_{O4}$ , with a recessive null allele,  $t_{O4}$ .

The present study provides evidence that the activity visualized by the nonspecific tetrazolium oxidase stain (actually, an artifact in the electron transfer staining system used to detect dehydrogenases) is actually catalyzed by superoxide dismutase isoenzymes. Since the name tetrazolium oxidase has fallen into disuse in isozyme literature, we conclude that the proteins previously referred to as tetrazolium oxidases should be referred to correctly as superoxide dismutases, EC 1.15.1.1. Further, in accordance with guidelines for assigning gene symbols to isoenzyme loci, we propose that the To4 locus and alleles be changed from To4 and to4 to SOD and sod. The inheritance of the second SOD variant, affecting the mobility of bands 8 and 9, has not been reported.

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# 2) Inheritance of a miniature mutant in soybean,

Mutant T251 was found by Dr. R. L. Bernard at the University of Illinois in an  $F_2$  progeny from the cross 'Harosoy'  $^5 \times$  T139. (T139 is a yellow mutant found in 'Illini' in 1936.) T251 is maintained in the Soybean Genetic Type Collection as the heterozygote T251H because of its low seed set. It is characterized by its short and slender stature. It has fewer nodes, shorter internodes, and smaller leaves than do normal plants, but internode length is greater than that of the dwarf mutants. Therefore, its growth type is reduced, resulting in the phenotype of a miniature plant.

Experiments were conducted from 1978 to 1981 at Iowa State University in order to determine the inheritance of T251. Two different crosses were performed: 'Minsoy' x T251, and 'Clark' T/T x T251. Minsoy carries the  $fr_1$  recessive allele for the absence of root fluorescence and the Pb allele for sharp pubescence tip. Clark T/T is an isoline of 'Clark' carrying a homozygous translocation from G. soja PI 101,404B. Clark T/T also carries the T allele for tawny pubescence. T251 is  $Fr_1$  pb t and carries the normal soybean chromosome complement. Homozygous recessive plants of T251 were used in the crosses.

For the Minsoy x T251 cross,  $F_2$  seed were germinated on paper towels for root fluorescence classification, then transplanted into a sandbench in the greenhouse for evaluation of plant height and pubescence tip.

For the Clark T/T x T251 cross, the  $\rm F_2$  plants were grown in the field and each normal stature plant was classified at maturity as homozygous (fertile) or heterozygous (semi-sterile) for the translocation, and harvested separately. The pubescence color was recorded at the same time. Twenty seed from each plant were then grown in the sandbench in the greenhouse to determine if the  $\rm F_2$  plant was heterozygous or homozygous dominant for the gene controlling plant height. Because of the poor viability of the mutant in the field, it was not possible to determine the segregation ratios at the  $\rm F_2$  generation.

The F<sub>2</sub> population from the Minsoy x T251 cross consisted of 1505 normal and 532 miniature types, which fits a 3:1 ratio ( $\chi^2$  = 1.35; P > 0.10). For the Clark T/T x T251 cross, 453 rows segregated for the miniature trait, and 249 were uniformly normal. This fits an expected 2:1 ratio ( $\chi^2$  = 1.44; P > 0.10). Those data, therefore, suggest that the miniature trait is controlled by a single recessive gene.

Because of the morphological difference between T251 and the dwarf mutants  $df_2$ ,  $df_3$ ,  $df_4$ , and  $df_5$ , it was not considered necessary to perform an allelism test with those mutants, and we, therefore, propose for the T251 mutation the new gene symbol mn (for "miniature").

Table 1. Linkage tests from soybean crosses Minsoy x T251 and Clark T/T x T251

		Minsoy >	T251	Total	χ <sup>2</sup>
$Fr_1$ Mn	$Fr_1$ mn	fr <sub>1</sub> Mn	$fr_1$ mn		
813	277	264	86	1440	0.099
Pb Mn	Pb mn	pb Mn	pb mn		
1096	395	406	137	2037	0.406
		Clark T/T	x T251		
F*Mnmn	F MnMn	$\mathit{SS}^{\dagger}\mathit{Mnmn}$	SS MnMn		
219	110	234	139	702	1.121
T Mnmn	T MnMn	tt Mnmn	tt MnMn		
333	192	120	57	702	1.103

<sup>\*</sup> F = fertile (homozygous for the translocation)

As seen in Table 1, an independence chi-square was performed between the mn gene and all other genes segregating from the crosses. No linkage was detected between mn and any of the genes  $fr_1$ , Pb, or T. Also, mn was not found to be linked to the breakpoint of the translocation from PI 101,404B.

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<sup>†</sup>SS = semi-sterile (heterozygous for the translocation).

## 3) Genetic studies with T263.

We reported inheritance studies and linkage tests with T263 (Palmer, 1977), a line carrying a gene for dwarfness. We had not completed allelism tests with the other available dwarfs,  $df_2$ ,  $df_3$ , and  $df_4$ , at that time. In this report, we give the allelism test results, as well as results of linkage tests of T263 with  $y_{13}$ ,  $y_{12}$ , G, T, and a chromosome interchange from PI 101,404B (Glycine soja). On the basis of allelism test results, the Soybean Genetics Committee has assigned the symbol  $df_5$ .

Crosses of T263 with the other dwarfs were made and the  $F_1$  plants grown in the greenhouse. The  $F_2$  seed were planted in the field at Ames at 7 seed per meter of row to allow for less competition between tall plants and dwarf plants. Dwarf plants were tagged at flowering and examined again at maturity. No attempt was made to distinguish between the dwarf phenotypes within an  $F_2$  population. About 80 tall  $F_2$  plants and about 20 dwarf  $F_2$  plants from each allelism test were threshed individually. Twenty-five seed were planted as  $F_2$  plant-progeny rows for evaluation. The relationship of observed and expected ratios were evaluated with the standard chi-square test for goodness of fit.

In linkage tests, a 'Clark' isoline homozygous for a chromosome interchange was used as the female parent and T263 plants as male parent. The F<sub>1</sub> plants were semisterile as expected and the F<sub>2</sub> plants were classified for tall/dwarf and tawny/gray but not for semisterility. It is difficult to determine if a dwarf plant is fertile or semisterile in a population segregating tall plants and dwarf plants. Fertile tall F<sub>2</sub> plants and semisterile tall F<sub>2</sub> plants were threshed individually, seed planted, and evaluated as F<sub>2</sub> plantprogeny rows the next year. The F<sub>3</sub> plants identifed F<sub>2</sub> genotypes; recombination value between  $df_5$  and interchange breakpoint was calculated according to Shands (1964).

 $F_2$  linkage tests from crosses between T263 and various mutants are presented using the general relationship that a = XY, b = Xy, c = xY, and d = xy for the gene pairs listed in the form of Xx and Yy. Percentage recombination was obtained from the ratio of products (Immer and Henderson, 1943).

In the allelism tests, all  $F_1$  plants were tall and the observed  $F_2$  ratio fit the expected 9 tall:7 dwarf ratio in each case (Table 1). Progeny of dwarf  $F_2$  plants bred true for dwarf in the  $F_3$ · Progeny of tall  $F_2$  plants could be classified as nonsegregating, segregating 3 tall:1 dwarf, and segregating 9 tall:7 dwarf. The observed ratio fit the expected ratio in each case (Table 1). The  $F_1$ ,  $F_2$ , and  $F_3$  data confirm the conclusion that the dwarf gene in T263 is different from  $df_2$ ,  $df_3$ , and  $df_4$ .

In the T263 x homozygous chromosome interchange cross, the  $\rm F_2$  plant-progeny rows identified  $\rm F_2$  genotypes  $\it Df_5$   $\it Df_5$  and  $\it Df_5$   $\it df_5$ . Percentage recombination between  $\it df_5$  and the interchange breakpoint was about 47.6 ± 2.1

T263 had been crossed to trisomics A, B, and C; and  $F_2$  plants (tall and dwarf) had been tagged for flower color and pubescence color (Palmer, 1977). Trait  $df_5$  was not located on trisomics A, B, or C, nor was it linked to flower color. It was linked to T t, of linkage group 1 with 15.4 percent recombination (Palmer, 1977).

Expected and observed F2 phenotypic and F2 genotypic ratios from soybean crosses between dwarf plants ( $df_5$   $df_5$ ) and dwarf plants  $df_2$   $df_3$ ,  $df_3$  and  $df_4$   $df_4$ , respectively Table 1.

			7	2		7			
Cross and phenotypes	No. F2 plants	x <sup>2</sup> 9:7	P No	No. F <sub>2</sub> plant-progeny rows	t-progeny	r rows	x <sup>2</sup> 1:4:4	Ф	No. all dwarfs
$df_2 df_2 \times df_5 df_5$		0.43	>0.95	Not seg.	3:1	9:7			
Tall	254			∞	32	31	0.02	0.99	
Dwarf	210								20
$df_5 df_5 \times df_3 df_3$		0.33	>0.90						
Tall	86			7	32	35	0.37	>0.75	
Dwarf	83								23
$df_{4}$ $df_{4}$ x $df_{5}$ $df_{5}$ and		0.39	0.95						
$df_5 df_5 \times df_4 df_4$									
Tall	231			6	38	33	0.36	>0.75	
Dwarf	191								22

Table 2. Genotypic classification for the  $df_5$  gene among fertile plants and semisterile F2 soybean plants from crosses between  $df_5$   $df_5$  plants and a homozygous chromosome interchange

		,
Genotype	Fertile *	Semisterile*
Df <sub>5</sub> Df <sub>5</sub>	43	47
$^{Df}_{5}$ $^{df}_{5}$	87	96

<sup>&</sup>quot;Within each fertility classification, data represent number of  $F_2$  plant-progeny rows evaluated.

Table 3. F $_2$  linkage tests from crosses between dwarf plants ( $df_5$   $df_5$ ) with various soybean mutants

	Genei	al phen	otypic c	lasses			Linkage
Genes	а	Ъ	С	d	Sum	% K±	phase*
Y <sub>13</sub> y <sub>13</sub> Df <sub>5</sub> df <sub>5</sub>	270	89	105	32	496	<u>49</u> ±3.4	R
$G$ $g$ $Df_5$ $df_5$	252	99	91	34	476	<u>51</u> ±3.5	С
<sup>Y</sup> 12 <sup>y</sup> 12 <sup>Df</sup> 5 <sup>df</sup> 5	1144	531	528	0	2203	<u>0</u> ±2.13	R
$T_1$ $t_1$ $Df_5$ $df_5$	1530	142	156	375	2203	14.5±0.81	С

<sup>\*</sup>R = repulsion and C = coupling.

In additional linkage tests,  $df_5$  was crossed to  $y_{13}$  and to G. Both of these mutants were independent of  $df_5$  (Table 3). T263 was crossed to  $y_{12}$  of Linkage Group 1. In the F<sub>2</sub> no  $df_5$   $y_{12}$  plants were identified. All tall yellow F<sub>2</sub> plants and dwarf green F<sub>2</sub> plants were threshed individually and will be evaluated as F<sub>2</sub> plant-progeny rows. R. I. Buzzell (Agriculture Canada) and I are working with  $df_5$ ,  $y_{12}$  and other mutants of Linkage Group 1 to determine gene order.

The F<sub>2</sub> plants in the chromosome interchange experiment provided us with additional  $Df_5$   $df_5$  -  $T_1$   $t_1$  linkage data. The percentage recombination was about 14.5±0.81 which agrees well with our previous value of 15.4 ± 1.0. The results with  $y_{12}$  and  $T_1$  confirm the conclusion that  $df_5$  is in linkage group 1.

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245

### 4) Linkage Group 12//

Weiss (1970a, b, c, d, e) described linkage groups 1 to 7 in soybean. Buzzell (1974, 1979) and Palmer (1977, 1984) have characterized further linkage group 1. Linkage group 8 was reported by Buzzell et al. (1977) and described further by Palmer and Kaul (1983), Sadanaga (1983) and Sadanaga and Grindeland (1984). Linkage groups 9, 10, and 11 were reported by Hildebrand et al. (1980), Kilen and Barrentine (1983), and Devine et al. (1983), respectively.

Broich et al. (1978) noted linkage between seed coat peroxidase level (ep) and nonfluorescent roots (fr-1). The purpose of this report is to present additional data on this linkage.

Linkage was calculated by the product method as described by Immer and Henderson (1943), where a = number of individuals carrying dominant alleles at both loci (A - B -), b = number of individuals carrying a dominant allele only for the A locus, c = number of individuals carrying a dominant allele only for the B locus, and d = number of individuals homozygous recessive at both loci (Table 1).

Ep has been found independent of W-1, e-3, fg-1, fg-2, and fg-3 (Buzzell et al., 1974) and f (Albertsen et al., 1983). Fr-1 was independent of rj-1, Rj-2, and P (Devine et al., 1983). We suggest that ep and fr-1 are loosely linked and define linkage group 12.

Crosses, alleles,  $\mathbb{F}_2$  progeny distributions and calculated recombination relationships for soybean linkage tests Table 1.

t + 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		Numb	Number of F, plants	ants		, r
ratetics and attetes	а	р	J O	q	Total	% and SE
Minsoy x Hark						
ep ep fr-1 fr-1 Ep Ep Fr-1 Fr-1	240	62	76	36	414	41.6 ± 3.3
Minsoy x T239						
ep ep fr-1 fr-1 Ep Ep Fr-1 Fr-1	398	113	127	61	669	42.7 ± 2.6
Minsoy x Trisomic C						
ep ep fr-1 fr-1 Ep Ep Fr-1 Fr-1	172	50	87	22	292	43.7 ± 4.9
Total	810	225	251	119	1405	42.6 ± 1.8

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5) Molecular analysis of organelle genomes of a cytoplasmically inherited mutant of soybean.

Very little is known about the physical and molecular organization of the genomes of plant organelles. Unlike the genes of a nuclear genome, which can be recombined by selective matings and mapped by recombinational events, the genes on an organelle genome are usually inherited as a unit, solely through a single parent. Therefore, when a uniparentally inherited mutation is observed, it is usually not possible to determine the location of the gene relative to other genes on the organelle genome, nor is it usually possible to determine genetically the organelle genome responsible for the mutant phenotype.

Provided that normal and mutant plants are sibs, we can assume that any difference between "normal" and "mutant" DNA is probably directly related to the molecular lesion causing the mutant phenotype. Thus, if we can screen normal and mutant organelle DNAs and somehow identify the molecular lesion associated with the mutant, we will have a molecular "tag" by which we can identify the coding sequence responsible for the mutant phenotype.

Palmer and Mascia (1980) reported the existence of a cytoplasmically inherited foliar mutant in soybean. This mutant is assigned Genetic Type Collection Number T275, and gene symbol  $cyt-Y_2$ .  $cyt-Y_2$  arose as a chimeric plant and subsequent selfings produced progeny that were yellow  $(cyt-Y_2)$ , green  $(cyt-G_2)$ , and chimeras. Therefore,  $cyt-Y_2$  and  $cyt-G_2$  are sibs and any sequence heterogeneity between the two would be indicative of the molecular event causing the mutant phenotype.

Our objective in this research was to isolate organelle DNA from normal and mutant plants, digest the DNA with a variety of restriction endonucleases, electrophorese the restricted DNA and analyze the restriction-fragment patterns for restriction-fragment size polymorphisms. In this way, we hoped to locate the mutation responsible for the  $cyt-Y_2$  phenotype to either the mitochondrial or the chloroplast genome and to gain information concerning the nature of the molecular lesion causing the mutant phenotype.

Materials and methods: Seed for the organelle-DNA comparisons of  $cyt-Y_2$  and  $cyt-G_2$  was increased in field plots at Ames, Iowa, and Isabela, Puerto Rico.

Isolation of mtDNA was carried out according to the procedure of Sisson et al. (1978) with the exception that dialysis was against low-TE buffer (0.01 M Tris, 0.005 M Na $_2$  EDTA, pH 8.0) and deproteinization was accomplished with phenol extractions followed by ether washes. ctDNA was isolated according to our rapid isolation procedure (Shoemaker et al., 1984).

mtDNA was digested with restriction endonucleases BamH I, EcoR I, Hind III, Kpn I, Sma I, and Xho I. ctDNA was digested with restriction endonucleases Ava I, Cla I, BamH I, Hind III, Xho I, and EcoR I. DNA was electrophoresed in 0.7% agarose gels made up in 90 mM Tris, 90 mM boric acid, and 2.5 mM Na $_2$  EDTA. Electrophoresis was carried out at room temperature for 17 hours at 40 V. ctDNA was electrophoresed in 0.8% agarose gels made up in 40 mM Trisacetate and 2 mM Na $_2$  EDTA. Electrophoresis was conducted at room temperature for 18 hours at 50 V. Gels were stained in distilled water containing 0.5  $\mu\text{m/ml}$  ethidium bromide and were photographed over short-wave ultra-violet light using an MP-4 camera with UV filter and Type 665 film.

Results and discussion: We observed no restriction-fragment polymorphism between mtDNAs or ctDNAs of  $cyt-Y_2$  and  $cyt-G_2$  comparisons. Therefore, we did not identify the organelle genome in which the mutation is located. Our results do, however, provide some valuable information concerning the nature of the molecular lesion causing the  $cyt-Y_2$  phenotype.

Restriction endonucleases recognize and cleave a 4-8 base pair sequence of double-stranded DNA (Smith, 1979). The absence of restriction-fragment polymorphism tells us that the  $cyt-Y_2$  phenotype is not the result of a base change in the recognition sequence of one of the enzymes used in this study. From the absence of restriction fragment polymorphism, we also know that the  $cyt-Y_2$  phenotype is not the result of a large addition or deletion, a translocation, or an inversion asymmetrically involving a restriction site. We can conclude that the  $cyt-Y_2$  phenotype probably results from a simple point-mutation. The normal chloroplast ultrastructure of  $cyt-Y_2$ , and its ability to develop nearnormal pigment levels, might suggest that the point-mutation is in a regulatory portion of the organelle genome, rather than in a structural gene. This information may provide physiologists valuable insight for the analysis of the biochemistry associated with chloroplast development and chlorophyll biosynthesis in the  $cyt-Y_2$  mutant.

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### 6) Is the $ms_4$ male-sterile mutant partially fertile?

The  $ms_4$  male-sterile mutant is inherited as a recessive allele. Plants homozygous for  $ms_4$  are male-sterile; heterozygotes are completely male-fertile. Male-sterility in the  $ms_4$  system results from the absence of cytokinesis following telophase II of microsporogenesis (Delannay and Palmer, 1982). A four-nucleate structure (coenocytic microspore) results that forms a pollen-like wall but does not seem capable of effecting fertilization. However, in many anthers, a delayed cytokinesis may occur (i.e., after pollen-wall deposition). This may result in tetrad-like clusters of cells resembling pollen grains. Delannay and Palmer raised the possibility that these structures may lead to infrequent self-pollination in "male-sterile" individuals. They noted that 75% of the seed harvested from  $ms_4$  male-sterile plants gave male-sterile progeny. Field-grown  $ms_4$  male-sterile plants often set up to 100 seed per plant, much higher than normal for other male-sterile plants grown in Iowa. This report summarizes current efforts to determine whether plants homozygous for  $ms_4$  can yield progeny via selfing.

In 1982, seed was harvested at random from five populations of malesterile plants grown in the field at Ames, Iowa. The  $ms_1$ ,  $ms_2$ ,  $ms_3$ ,  $ms_4$ , and Beeson mutants were used. The latter is nonallelic to  $ms_1$ ,  $ms_2$ ,  $ms_3$ , and  $ms_4$ . We currently are conducting allelism tests with the  $ms_5$  mutant. The maximum frequency of male-sterile progeny one can expect in seed from malesterile plants is 50%. This would occur only if male parents were always heterozygous for the locus in question. Pollination in the experiment was uncontrolled; any significant increase in the frequency of male-sterile progeny above the 50% level must be due to some mechanism other than outcrossing. Results (Table 1) show that only in the case of  $ms_4$  was a frequency of malesterile individuals in excess of 50% attained. This led us to suspect that some form of selfing was occurring.

Table 1. Frequency of fertile and male-sterile individuals in progeny of field-grown male-sterile plants

Parental	Proge	eny	
genotype	Fertile	Sterile	Percent sterile
$ms_1$ $ms_1$	69	18	20.1
$ms_2$ $ms_2$	47	32	40.5
$ms_3$ $ms_3$	51	18	26.1
$ms_4$ $ms_4$	4	105	96.3
Beeson	33	32	49.2

In addition to the field-grown individuals, 65 progeny of field-grown  $ms_4$  male-sterile plants were greenhouse-grown in the summer of 1983. Of the 65, 62 were male sterile. We wanted to determine whether  $ms_4$   $ms_4$  individuals would set seed in the absence of pollinators. Of the 62  $ms_4$  male-sterile plants, 23 produced pods in the greenhouse. The number of seed ranged from one to eight per plant. From the 23  $ms_4$  plants, 60 seed were obtained. Forty of these germinated and grew to maturity; 38 were male-sterile. The over-abundance of male-sterile progeny from these plants supports the hypothesis of self-pollination by  $ms_4$   $ms_4$  individuals. Six individuals of the genotype  $ms_2$   $ms_2$  were included in the same house as controls. Two of the  $ms_2$  plants produced a single seed each. Progeny of the  $ms_2$  plants were both male fertile, indicating that pollen vectors must have gained access to the greenhouse.

We have begun growth-chamber experiments in an attempt to determine whether the environment may influence the tendency toward selfing. Plants were grown under 16-hour daylengths for 3 weeks; daylength was then reduced to 14 hours. Both fluorescent and incandescent lights were used. Results of one trial using three temperature regimes are given in Table 2. The medium temperature  $(29^{\circ}/23^{\circ}\text{C})$  appears to be optimal for seed production. All male-sterile plants set seed under this temperature regime. In the high temperature environment, not a single male-sterile plant produced seed. Seed was produced by male-sterile plants grown in the cool chamber, but the number, expressed both as mean number of seed per plant and as a percentage of fertile seed set, was less than in the medium environment.

Table 2. Seed production by male-sterile  $(ms_4)$  and male-fertile plants grown under three temperature regimes

Temperature (°C) (day/night)	No. of sterile plants	Seeds per plant (mean)	No. of fertile plants	Seeds per plant (mean)	Sterile yield Fertile yield
24°/21°	6	0.67	3	25.7	2.6%
29°/23°	6	6.50	5	167.0	3.9%
35°/32°	10	0.0	2	64.0	0.0

The mechanism of male-sterility in  $\mathit{ms}_4$  is similar to that of  $\mathit{ms}_1$  (Albertsen and Palmer, 1979). The  $\mathit{ms}_1$  locus is known to have a pleiotropic effect on female reproduction. A high frequency of polyploid and polyembryonic seedlings is recovered in the progeny of  $\mathit{ms}_1$   $\mathit{ms}_1$  plants (Kenworthy et al., 1973; Beversdorf and Bingham, 1977). We have yet to find a similar response in  $\mathit{ms}_4$ . Over 300 seedlings were screened for polyembryony, with negative results. Chromosome numbers were established for 40 progeny of male-sterile plants; all had the normal diploid component of  $2\mathrm{n}{=}40$ .

There are three possible means by which male-sterile individuals can set seed in the absence of cross-pollination. The pollen-like structures noted by Delannay and Palmer may be capable of pollination and fertilization. In some anthers, however, cytokinesis may occur as in fertile anthers, resulting in normal pollen formation. We cannot rule out this possibility; however, we have never observed normal cytokinesis in the hundreds of anthers examined. A third possibility is that apomixis is occurring. We are presently using the chlorophyll-deficient mutant  $y_{11}$  in an attempt to discriminate between apomictic reproduction and self-pollination as the origin of the seed produced by male-sterile plants.

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# 7) Evaluation of Glycine soja from The People's Republic of China and the USSR.

A chromosome interchange was suspected by Williams (1948) in a cross involving *Glycine soja* Sieb. and Zucc. PI 101404B. This interchange was confirmed by Palmer and Heer (n.d.).

The objective of the present study was to search for chromosome interchanges among G. soja accessions from The People's Republic of China and the USSR.

Crosses were made between these accessions and cultivars of G. max, which had noninterchange chromosomes and were designated N/N for normal chromosome structure. Plants were classified for pollen fertility by using a solution of  $I_2KI$ . Fertile pollen grains were plump and stained red-brown; aborted pollen grains were shrunken, collapsed, and unstained or only very lightly stained. This latter condition is termed semisterility since the sterility is about 50%. Percentage of ovule abortion was calculated by dividing the number of ovule abortions by the total number of mature seed, seed abortions, and ovule abortions.

The People's Republic of China: Sixteen accessions of G. soja were crossed to G. max (Table 1). Only two recent accessions gave fertile  $F_1$  hybrids; the remaining 14 gave  $F_1$  hybrids with about 50% pollen sterility. In the four  $F_1$  hybrids examined, ovule sterility agreed with the pollen sterility.  $F_2$  plants from these same four  $F_1$  hybrids gave both the expected fertile plants and the semisterile plants. These 14 accessions were considered to have homozygous interchange chromosomes (T/T).

These 14 accessions were crossed in various combinations, and pollen sterility and, in some cases, ovule sterility were determined (Table 2). All these accessions have the identical chromosome interchange.

<u>USSR</u>: Twenty-one accessions of *G. soja* were crossed to *G. max* (Table 3). Two accessions were found to be a mixture of chromosome structure genotypes. These accessions are PI 423989B and PI 423990A. The -1 and -2 designations in Tables 3, 4, and 5 are ours, and not part of the official Plant Introduction number. Only two accessions had N/N chromosome structure, while 17 were considered to have T/T chromosome structure.

These 21 accessions, plus others from the USSR, were intercrossed. Based upon pollen sterility and ovule sterility, these accessions were considered to have interchange chromosomes (Table 4), or normal chromosomes (Table 5). We have identified additional PIs 423990B, 423992, 423995, and 423999B as having T/T chromosome structure. The data in Table 5 offer confirmation of the N/N or T/T chromosome structure for many of the USSR G. soja accessions. All T/T accessions have the identical chromosome interchange. The only cross of two homozygous normal chromosome accessions (PI 423990A-2 x PI 424001) gave male- and female-fertile  $F_1$  and  $F_2$  plants (data not given).

The People's Republic of China - USSR Intercrosses: We made eight different intercrosses between accessions from these two countries that had T/T chromosome structure (Table 6). All  $F_1$  hybrids were fertile. This indicates that all the accessions from The People's Republic of China and the USSR that have homozygous interchange chromosomes have the identical chromosome structure. In fact, the majority of accessions from these countries have this interchange.

Crossing studies with G. soja accessions from Korea and Japan and G. max cultivars have been initiated. Preliminary results indicate that these accessions have a low frequency of homozygous interchange chromosomes (Delannay et al., 1982).

Table 1. Glycine soja from The People's Republic of China: Pollen and ovule sterility of F1 and F2 plants in crosses with cultivated soybean G. max as female parent

Plant	No. of	Sterilit	y (%)	No. of	Sterility (%)
Introduction	Fl plants	Female	Male	F2 plants	Male
65549	5*	47.2	55.1	16 10	4.4 52.5
101404A	2*	48.1	53.3	4 6	3.2 51.9
101404B	2 <b>*</b>	50.0	50.4	15 18	5.1 50.9
135624	4 <b>*</b>	49.3	51.0	6 4	7.1 53.2
391587	2		51.2		
407288	8*		50.5		
407290	1		53.1		
407291	1		51.8		
407292	1		52.3		
407294	4*		53.5		
407296	2		52.3		
407299	2		50.0		
407301	1		47.8		
407302	2		48.5		
468916	2		2.1		
468918	2		1.7		

<sup>\*</sup>Includes reciprocal cross.

Pollen and ovule sterility of Fl and F2 Table 2. Glycine soja from The People's Republic of China: plants in crosses between homozygous interchange plants

Plant Number Introduction FI pla 101404A 4* 101404B 2* 135624 4* 135624 4* 135624 6* 101404B 2 101404B 2 101404B 2 101404B 2 407290 2 407290 2 407291 2 407292 2 407294 2 407299 2 407301 2 407301 2							
101404A       4*       3.9       3.2       10         101404B       2*       6.0       1.9       5         135624       4*       7.9       2.1       10         135624       4*       5.8       2.4       10         101404A       2       4.1       1.8       5         101404B       2       10.3       1.7       5         101404B       2       11.7       2.1       5         101404B       2       11.2       1.4       5         10524       2       11.2       1.4       5         40728B       4       2.6       1.5       5         40729C       2       1.1       2.2       4.1         40729C       2       1.9       4.1       4.1         40729C       2       2.2       4.1       4.1         40729C       2       2.2       4.1       4.1         40729C       2       2.2       2.8       4.1         40729C       2       2.2       2.5       2.5         40729C       2       2.2       2.5       3.1         40730C       2       2.2       2.2 <th>Plant Introduction</th> <th>Plant Introduction</th> <th>Number of Fl plants</th> <th>Sterilit</th> <th>(%) Ma 1</th> <th>Number of F2 plants</th> <th></th>	Plant Introduction	Plant Introduction	Number of Fl plants	Sterilit	(%) Ma 1	Number of F2 plants	
101404B       2*       6.0       1.9       5         135624       4*       7.9       2.1       10         135624       4*       7.9       2.1       10         101404A       2       10.3       1.7       5         101404B       2       10.5       2.1       5         101404B       2       11.7       2.1       5         10405B       2       11.2       1.4       5         40728B       4       5.6       1.5       5         407290       2       1.5       5         407291       2       4.1       5         407292       2       4.1       5         407294       2       1.9       2.2         407295       2       2.2       4.1         407296       2       4.1       2.8         407299       2       2.2       4.1         407296       2       2.2       2.8         407299       2       2.2       2.8         407299       2       2.2       3.1         407299       2       2.5       3.1         407390       2       2.2	65249	101404A	*7			10	1.6
135624       4*       7.9       2.1       10         135624       4*       5.8       2.4       10         101404A       2       4.1       1.8       5         101404B       2       10.5       2.1       5         101404B       2       11.7       2.1       5         135624       2       11.2       1.4       5         40728B       4       2.5       1.5       5         407290       2       5.6       1.5       5         407291       2       4.1       1.9       7         407294       2       2.8       2.8       4         407299       2       2.8       2.8       2.8         407299       2       2.8       2.8       2.8         407299       2       3.1       2.5       3.1         407301       2       2.5       3.1       3.1         407302       2       3.1       3.1       3.1         407301       2       2.5       3.1       3.1         407302       2       3.1       3.1       3.1         407302       2       3.2       3.1	62249	101404B	2*	0.9	1.9	5	
135624       4*       5.8       2.4       10         101404A       2       4.1       1.8       5         101404B       2       10.5       2.3       5         101404B       2       11.7       2.1       5         101404B       2       11.7       2.1       5         101524       2       11.5       1.4       5         40728       4       5.6       1.5       5         407290       2       2.2       4.1       5         407294       2       2.8       2.8       4.1         407295       2       2.8       2.8       4.1         407301       2       2.5       2.5       2.5         407301       2       2.5       2.5       2.5         407302       2       2.5       2.5       2.5         407302       2       2.5       2.5       2.5         407302       2       2.5       2.5       2.5         407302       2       2.5       2.5       2.5         407302       2       2.5       2.5       2.5         407302       2       2.5       2.5	65249	135624	*7		2.1	10	
101404A       2       4.1       1.8       5         101404B       2       10.5       2.3       5         101404A       2       11.7       2.1       5         101404B       2       11.2       1.4       5         135624       2       1.5       5         407288       4       3.2       5         407290       2       4.1       5         407291       2       4.1       1.9         407294       2       2.8       2.8         407296       2       2.8       2.8         407297       2       2.5       2.5         407301       2       2.5       2.5         407302       2       2.5       2.5         407304       2       2.5       2.5         407307       2       2.5       2.5         407307       2       2.5       3.1         407307       2       2.5       3.1	101404A	135624	*7	5.8	2.4	10	
65549       2       10.5       2.3       5         101404A       2       11.7       2.1       5         101404B       2       11.2       1.4       5         135624       2       11.2       1.4       5         391587       2       5.6       1.5       5         407288       4       2.2       4.1       5         407290       2       4.1       1.9       4.1         407291       2       2.8       2.8       2.8         407294       2       2.8       2.8       2.8         407299       2       3.1       2.5       4.0         407301       2       3.1       1.9       2.5         407302       2       2.5       2.5       2.5	101404B	101404A	2	4.1	1.8	5	2.0
65549       2       10.5       2.3       5         101404A       2       11.7       2.1       5         101404B       2       11.2       1.4       5         135624       2       5.6       1.5       5         407288       4       2.2       5         407290       2       4.1       6         407291       2       4.1       6         407292       2       2.8       2.8         407299       2       2.5       2.5         407301       2       3.1       1.9         407302       2       2.5       2.5	135624	101404B	2			5	
101404B       2       11.7       2.1       5         101404B       2       11.2       1.4       5         135624       2       5.6       1.5       5         40728B       4       2.2       5.2       7.2         407291       2       4.1       1.9       7.9         407294       2       2.8       2.8       2.8         407296       2       2.5       3.1         407301       2       3.1       1.9         407302       2       3.1       1.9	1014048**	65549	2	10.5		5	
101404B       2       11.2       1.4       5       4         135624       2       5.6       1.5       5       1         391587       2       3.2       5       1         407288       4       4.1       4.1       4.1         407291       2       1.9       4.1       4.1         407294       2       2.8       2.8       4.2         407296       2       2.5       3.1       4.1         407301       2       3.1       1.9         407302       2       2.5       2.5	0+ 9+	101404A	2	11.7		5	2.0
135624       2       5.6       1.5       5       1         391587       2       3.2       3.2         407288       4       4.1       4.1         407291       2       1.9       3.2         407294       2       2.8       2.8         407296       2       2.5       3.1         407299       2       3.1       1.9         407301       2       3.1       1.9	g- g-	101404B	2		1.4	5	
391587       2         407288       4         407290       2         407292       2         407294       2         407296       2         407299       2         407301       2         407302       2		135624	2		1.5	5	
407288       4         407290       2         407292       2         407294       2         407296       2         407299       2         407301       2         407302       2		391587	2				
407290       2       4         407291       2       3         407294       2       2         407296       2       2         407299       2       3         407301       2       3         407302       2       3	01- 60-	407288	7				
407291       2       1         407292       2       3         407296       2       2         407299       2       3         407301       2       1         407302       2       2	e- *-	407290	2		4.1		
407292       2         407294       2         407296       2         407299       2         407301       2         407302       2	Ē	407291	2		1.9		
407294       2         407296       2         407299       2         407301       2         407302       2	60- 60-	407292	2		3.2		
407296       2       2         407299       2       3         407301       2       1         407302       2       2	-	407294	2		2.8		
407299       2       3.         407301       2       1.         407302       2       2.	4.2	407296	2		2.5		
407301     2     1.       407302     2     2.	Give Give	407299	2		3.1		
407302 2 2.		407301	2		1.9		
	=	407302	2				

\*Includes reciprocal crosses.

<sup>\*\*&#</sup>x27;Clark' near-isogenic line homozygous for a chromosome interchange from G. soja PI 101404B.

Table 3. Glycine soja from the USSR: Pollen and ovule sterility of F1 plants in crosses with cultivated soybean G. max as female parent

Plant	Number of	Sterilit	y (%) ———
Introduction	F1 plants	Female	Male
81762	4		51.2
326581	6*		6.6
326582A	2		52.9
342618B	2		51.4
342622A	8*		47.2
342622B	2		53.2
423988	2	48.4	53.3
423989A	1	53.1	55.1
423989B-1	5*	49.1	52.2
423989B-2	2	6.9	2.0
423990A-1	3	48.7	53.0
423990A-2	1	5.3	1.2
423991	3	49.7	53.1
423993	1	52.1	52.1
423994	2	48.7	49.0
423996	2	47.3	49.1
423997	1	49.0	45.0
423998	5*	46.9	50.6
423999A	1	49.0	52.3
424000	2	47.3	49.9
424001	5*	9.1	4.2
424002	3	47.4	53.0
424003	1	46.8	51.9

<sup>\*</sup>Includes reciprocal cross.

plants in crosses Pollen and owule sterility of Fl and F2 Glycine soja from the USSR: Table 4.

Introduction 342622A 423989B-1	Tanc	Number of	Sterility	Ey (%)	Number of	Sterility (%)
342622A 423989B-1	Introduction	Fl plants	Female	Male	F2 plants	Male
423989B-1	423998	3		1.2		
	423991		4.5		5	
423989B-1	424002		8.0	6.7	2	4.8
423990A-1	423991		1.3	3.1	5	6.4
423990A-1	423995			4.3	2	3.4
423990A-1	423997			2.7	2	4.4
423990A-1	424000			7.4	5	6.9
423990B	423997	$\vdash$			2	3.5
423991	423999B	2*		2.9	10	8.4
423991	424003	2*		3.0	10	3.4
423992	424991			2.7	5	5.7
423992	423994				5	5.6
423992	424999B	1	7.0		5	5.5
423992	424000	<u> </u>	7.3		5	9.7
423993	423995	-	11.0	1.8	5	6.4
423994	423991		3.3		5	7.7
423994	424003		9.4		5	4.8
423995	423992		6.8		5	3.9
423995	423996		6.3		5	7.8
423997	423991		11.3		5	4.3
423997	423998		8.3		5	6.5
423998	423991	1	11.4	0.7	5	5.7
423999A	423997		8.1	3.0	9	6.2
423999B	423994		7.3	2.1	5	7.2
423999B	423998		8.9	2.1	5	7.4
423999B	423999A	1	8.2	1.7	5	9.5
424000	423991	1	8.0	1.7	5	5.5
424000	423995	1	7.6	2.1	5	2.3
424000	423997	1	11.5	1.4	5	6.2

\*Includes reciprocal cross.

Table 5. Glycine soja from the USSR: Pollen and ovule sterility of Fl and F2 plants in crosses between homozygous interchange plants and homozygous normal-chromosome plants

Plant Intro.	Plant Intro.	No. of Fl plants	Sterility Female	(%) Male	No. of Sterility (%) F2 plants Male
423989B-2	423990A-1	1	48.8	53.1	2 7.8 3 50.2
423989B-2	423994	1	50.9	53.5	$ \frac{1}{1} \frac{7.6}{54.2}$
423989B-2	423998	1	50.3	53.0	<del> </del>
423989B-2	423999A	2*	50.5	56.7	8 6.3 7 51.9
423990A-1	424001	1	48.1	56.6	$\frac{1}{2}$ $\frac{1}{6.5}$ $\frac{54.9}{1}$
423990A-2	423992	1	52.5	55.4	$\frac{3}{2}$ $\frac{4.1}{53.2}$
423990A-2	423994	2*	49.7	49.9	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
423990A-2	423998	2*	49.7	52.0	5 5.6 5 54.3
423990A-2	423999A	2*	49.4	53.6	$\frac{14}{14} - \frac{3}{7.3} - \frac{1}{1}$
423993	423990A-2	1	49.0	51.8	$\frac{1}{1}$ $\frac{1}{4}$ $\frac{1}$
423997	423989B-2	1	51.3	50.7	$\frac{1}{1}$ $\frac{1}{4}$ $\frac{8.6}{53.4}$
424001	423991	1	48.8	51.1	$\frac{1}{4} = \frac{1}{4} = \frac{1}{9.7} = \frac{1}{9.7}$
424001	423992	1	48.6	52.7	$\frac{1}{2}$ $\frac{1}$
424001	423994	1	49.1	52.4	$\frac{3}{3}$ $\frac{2.6}{2}$ $\frac{49.2}{2}$
424001	423997	1	49.7	52.1	5 4.0 5 50.7
424001	423998	1	48.8	51.7	$\frac{2}{2}$ $\frac{2}{2}$ $\frac{2}{2}$ $\frac{5}{3}$ $\frac{48.0}{2}$
424001	423999A	1	48.6	52.5	$\frac{1}{4}$ $\frac{1}{4 \cdot 5}$ $\frac{1}{51 \cdot 2}$
424002	424001	1	49.4	52.6	$\frac{1}{7} - \frac{1}{7} - \frac{1}{5 \cdot 1} = \frac{1}{5 \cdot 1}$

<sup>\*</sup>Includes reciprocal cross.

Table 6. Glycine soja from The People's Republic of China and the USSR:
Pollen sterility of F1 plants in crosses between homozygous interchange plants

Plant Introduction	Plant Introduction	Number of Fl plants	Sterility (%) Male
101404B**	81762	2	3.3
101404B**	326581	2	1.9
101404B**	326582A	2	4.1
101404B**	342618B	2	1.9
101404B**	342622A	3	0.6
101404B**	342622B	2	2.6
101404B**	423998	3	4.1
407288	342622A	1	5.3

<sup>\*\*</sup>A Clark near-isogenic line homozygous for a chromosome interchange from Glycine soja PI 101404B.

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## 8) A possible interaction between $cyt-Y_3$ and $y_{20}-k_2$

Interactions of organelle genomes are a well-documented fact. Interaction can take the form of metabolite and energy exchange, co-production of enzyme sub-units, and co-production of membrane structural and organizational components (Wallace, 1982). Nucleo-cytoplasmic interaction also has been documented for various traits affecting vegetative and harvest indices (Robertson and Frey, 1984), cytoplasmic male sterility (Levings, 1983), and other agronomic characters (Harvey et al., 1972; Kihara, 1982).

Previous results have indicated that a nucleo-cytoplasmic interaction occurs between the soybean nuclear mutant  $y_{20}$ - $k_2$  (chlorophyll-deficient, tansaddle seed coat; Genetic Type Collection Number T253) and soybean cytoplasmic mutant  $cyt-Y_2$  (Palmer and de Cianzio, 1984). The nuclear mutant  $y_{20}-k_2$ 

is unique in that it is inherited as a single gene. Under field conditions, with  $y_{20}$ - $k_2$  in the presence of cyt- $Y_2$  cytoplasm, no  $k_2$  (tan-saddle seed coat) seed is found. With special care,  $y_{20}$ - $k_2/y_{20}$ - $k_2$  plants can be grown under greenhouse conditions. The combination cyt- $y_2$   $y_{20}$ - $k_2/y_{20}$ - $k_2$  is, therefore, a conditional lethal.

Our objective in this study was to determine if a nucleo-cytoplasmic interaction occurs between the soybean nuclear mutant  $y_{20}$ - $k_2$  and the soybean cytoplasmic mutant cyt- $Y_3$  (Genetic Type Collection Number T278). We crossed  $y_{20}$ - $k_2$  plants reciprocally with yellow branches of cyt- $Y_3$  chimeras. These crosses were advanced to the  $F_2$  for analysis. All progeny containing the cyt- $Y_3$  cytoplasm were grown in the greenhouse under reduced light conditions. The results of these crosses are shown in Table 1.

Table 1. Phenotypes observed in  $F_1$  and  $F_2$  generations from reciprocal soybean crosses of nuclear mutant  $y_{20}$ - $k_2$  and cyt- $Y_3$  chimera

			20 2	3
———Pa	rents	S ———		
Female		Male		
y <sub>20</sub> -k <sub>2</sub>	X	Chimera	22 green	1351 green, nonsaddled seed 344 yellow, saddled seed
Chimera	X	<sup>y</sup> 20 <sup>-k</sup> 2	23 yellow	87 yellow, nonsaddled seed 21 yellow, early-lethal
			5 green	332 green, nonsaddled seed 85 yellow, saddled seed
			9 chimera	

In crosses using  $y_{20}$ - $k_2$  as the female parent, we saw normal Mendelian segregation (3:1) of  $Y_{20}$ - $K_2$ : $y_{20}$ - $k_2$  in the F<sub>2</sub> generation. In the reciprocal cross, sorting-out and transmission of mutant plastids resulted in F<sub>1</sub> progeny that were yellow, green, or chimera. Among F<sub>2</sub> from the green plants, we again saw a 3:1 segregation of  $Y_{20}$ - $K_2$ : $y_{20}$ - $k_2$ . These results indicate that the mutant  $y_{20}$ - $k_2$  trait behaves normally in "normal" cytoplasm.

Among F<sub>2</sub> of the yellow segregants from the chimera X  $y_{20}$ - $k_2$  cross, we observed 87 yellow plants with nonsaddled seed, and 21 yellow plants that died at an early seedling stage. Since the ratio of nonlethal, nonsaddled plants to early-lethal plants was 3:1, we presume that early-lethality is the result of the presence of  $y_{20}$ - $k_2$ / $y_{20}$ - $k_2$  in cyt- $Y_3$  cytoplasm. These results are similar to those reported from the interaction between  $y_{20}$ - $k_2$  and cyt- $Y_3$  (Palmer and de Cianzio, 1984).

The mutant  $cyt-Y_3$  is an extremely weak mutant (Shoemaker et al., 1984) and it is possible that the additive effect of two chlorophyll mutations is simply creating a condition that is so detrimental to the soybean that the plant cannot survive. The question as to whether or not this situation is a true nucleo-cytoplasmic interaction or whether it is only a manifestation of an increased genetic load will only be answered by an in-depth physiological/biochemical analysis coupled with further detailed genetic studies.

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 $^{\it 245}$  SUMMARY OF LOCUS-TO-LOCUS LINKAGE DATA IN SOYBEAN  $_{\it //}$ 

$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			f		1						
89         105         32         496         49.0 †         3.4 †         R           99         91         34         476         51.0         3.5 †         C           299         336         113         1700         51.0         1.8         R           53         39         21         288         57.4         4.0         R           49         50         10         288         46.7         4.6         R           54         50         248         57.0         4.9         C           16         248         52.0         4.9         C           17         27         6         99         56.6         8.1         C           24         42         8         140         7.5         R           24         42         8         140         5.4         7.0         R           20         28         140         5.4         7.0         R           24         42         8         140         7.5         R           25         28         140         7.5         R           20         28         140         7.0         <	Genes		AB	henotypic Ab	CT	ab	Sum		S.E.	Phase	Reference
99         91         34         476         51.0         3.5         C           299         336         113         1700         51.0         1.8         R           53         39         21         288         46.7         4.0         R           54         50         10         288         46.7         4.6         R           54         50         10         288         46.7         4.6         R           54         50         248         52.0         4.9         C           54         54         20         248         52.0         4.9         C           17         27         6         99         56.6         8.1         C           24         42         6         140         7.5         R           24         42         8         140         40.6         7.0         R           25         28         140         40.6         7.0         R           26         28         140         6.0         7.0         R           27         45         11         131         48.7         6.7         R	5	y <sub>13</sub>	270		105	32	967	0	1 +	NA NA	25
299       336       113       1700       51.0       1.8       B         53       39       21       288       57.4       4.0       R         49       50       10       288       46.7       4.6       R         56       70       10       288       77.0       6.0       R         54       54       248       77.0       6.0       C         16       25       8       99       50.2       7.6       C         17       27       6       99       56.6       8.1       C         24       42       140       40.6       7.5       R         25       42       6       140       7.5       R         26       24       40.0       7.0       R         27       42       8       140       40.6       7.0       R         28       12       131       43.9       7.0       R       R         29       28       12       46.7       6.7       R       R         21       44       12       131       48.7       6.7       R       R         24       25 <td>5</td> <td>8</td> <td>252</td> <td></td> <td>91</td> <td>34</td> <td>476</td> <td>51.0</td> <td>3.5</td> <td>O</td> <td>25</td>	5	8	252		91	34	476	51.0	3.5	O	25
53       39       21       288       57.4       4.0       R         49       50       10       288       46.7       4.6       R         56       70       4       248       77.0       6.0       C         54       20       248       52.0       4.9       C         16       25       8       99       56.6       8.1       C         17       27       6       99       56.6       8.1       C         24       42       8       140       40.6       7.5       R         25       8       140       40.6       7.0       R         20       28       12       40.0       5.4       C         20       28       12       40.0       5.4       C         21       45       11       131       48.7       6.7       R         21       44       12       131       48.7       6.7       R         22       28       12       46.7       8       7.0       R       R         21       44       12       131       48.7       6.7       R       R	5	$I_{\mathcal{M}}$	952	2	336	113	1700	51.0	1.8	ĸ	23
49         50         10         288         46.7         4.6         R           56         70         4         248         77.0         6.0         C           54         54         20         248         77.0         6.0         C           16         25         8         99         50.2         7.6         C           17         27         6         99         56.6         8.1         C           24         42         8         140         40.6         7.5         R           25         44         6         140         40.6         7.5         R           26         28         140         40.6         7.0         R           27         8         140         40.6         7.0         R           29         11         131         43.9         7.0         R           44         12         131         48.7         6.7         R           51         44         12         79.5         R         R           52         70         79.5         R         R           6         74         78.5         79.5	7.	ms <sub>2</sub>	175		39	21	288	57.4	7.0	M	17
56         70         4         248         77.0         6.0         C           54         54         20         248         52.0         4.9         C           16         25         8         99         50.2         7.6         C           17         27         6         99         56.6         8.1         C           24         44         6         140         32.4         7.5         R           20         28         140         40.6         7.0         R           21         45         11         131         43.9         7.0         R           21         45         11         131         48.7         6.7         R           25         71         25         392         7.9.5         3.7         C	7	$V_{\mathcal{I}}$	179		20	10	288	46.7	9.4	R	17
54         54         20         248         52.0         4.9         6           16         25         8         99         50.2         7.6         C           17         27         6         99         56.6         8.1         C           32         44         6         140         32.4         7.5         R           24         42         8         140         40.6         7.0         R           20         28         12         155         40.0         5.4         C           21         45         11         131         48.7         6.7         R           15         12         131         48.7         6.7         R           75         71         25         392         7.95         3.7         C	. 02	ms <sub>2</sub>	118		70	7	248	77.0	0.9	U	17
16       25       8       99       50.2       7.6       C         17       27       6       99       56.6       8.1       C         32       44       6       140       32.4       7.5       R         24       42       8       140       40.6       7.5       R         20       28       12       155       40.0       5.4       C         21       45       11       131       48.7       6.7       R         15       12       131       48.7       6.7       R         15       12       131       48.7       6.7       R         15       12       131       48.7       6.7       R         15       13       25       392       79.5       3.7       C	. 2	$T_{M}$	120		54	20	248	52.0	6.4	U	17
17       27       6       99       56.6       8.1       C         32       44       6       140       32.4       7.5       R         24       42       8       140       40.6       7.0       R         20       28       12       155       40.0       5.4       C         21       45       11       131       43.9       7.0       R         15       44       12       131       48.7       6.7       R         75       71       25       392       7.9.5       3.7       C		$ms_{\mathcal{I}}$	50		25	$\infty$	66	50.2	7.6	U	17
32       44       6       140       32.4       7.5       R         24       42       8       140       40.6       7.0       R         20       28       12       155       40.0       5.4       C         21       45       11       131       43.9       7.0       R         15       44       12       131       48.7       6.7       R         75       71       25       392       7.9.5       3.7       C		$M_{I}$	67		27	9	66	56.6	8.1	U	17
24         42         8         140         40.6         7.0         R           20         28         12         155         40.0         5.4         C           21         45         11         131         43.9         7.0         R           15         44         12         131         48.7         6.7         R           75         71         25         392         7.9.5         3.7         C		$ms_1$	58		77	9	140	32.4	7.5	ĸ	1.7
20       28       12       155       40.0       5.4       C         21       45       11       131       43.9       7.0       R         15       44       12       131       48.7       6.7       R         75       71       25       392       79.5       3.7       C		$T_{\mathcal{M}}$	99		42	$\infty$	140	9.07	7.0	M	17
21     45     11     131     43.9     7.0     R       15     44     12     131     48.7     6.7     R       75     71     25     392     79.5     3.7     C		ep	95		28	12	155	0.04	5.4	C	ĸ
15     44     12     131     48.7     6.7     R       75     71     25     392     79.5     3.7     C		$ms_1$	54		45	11	131	43.9	7.0	R	17
75 71 25 392 749.5 3.7 C		$M_{\mathcal{I}}$	09	П	77		131	48.7	6.7	M	17
		$T_{M}$	221	7	71	25	392	76.6	3.7	Ö	3,4

Summary of locus-to-locus linkage data in soybean (continued)

Genes		AB PI	nenotypic Ab	classes — aB	ab	Sum	% R	S.E.	Phase	Reference
də	£	739	243	239	80	1301	51.0	2.0	M	
	1,2	146	42	41		240	51.0	3.5	C	5
ab	$M_{I}$	393	112	141	32	713	8.94	3.0	R	2
Ф	$T_{\mathcal{M}}$	236	62	75	22	395	48.5	3.7	C	3,4
J	ms <sub>1</sub>	101	101 36	38	12	187	48.3	5.6	ĸ	17
	$M_{\perp}$	855	211	287	98	1439	23.0	2.4	X	2,17
$f_{\mathcal{Q}}$	đt	270	68	82	29	500	1 67		ت	9
	E 2	131	50		11	236	> 55	5.2	) ()	3,7
	ep	96	28	28	7	156	> 55	7.9	O	æ
	$fg_2$	283	95	80	32	190	52.5	5.2	K	3,4
	$fg_3$	362	119	114	37	632	8.67	3.0	ĸ	3,3
	$fg_4$	200	71	69	17	357	56.0	4.2	C	8
	į	188	74	58	25	345	51.0	7.0	ĸ	Э
	p <sub>2</sub>	208	63	62	24	357	53.0	3.8	ĸ	3
	$\Gamma$	130	39	39	14	222	52.0	6.4	æ	3
	$W_{\mathcal{I}}$	248	78	74	29	429	8.97	3.4	C	3,8
	$M_{\mathcal{I}}$	92	22	34	6	141	0.64	7.9	æ	7

continued ...

Summary of locus-to-locus linkage data in soybean (continued)

		1	Phenotynic	20000						
Genes		AB	Ab	aB	ab	Sum	% R	S.E.	Phase	Reference
	e G	84	31	31	6	155	53.0	6.2	O	3
	$e^{D}$	212	65	69	25	371	47.5	3.8	C	3,6
	£93	398	125	134	67	902	47.8	2.7	C	3
	$fg_4$	121	48	37	15	221	50.3	5.1	M	3
	7,	182	7.5	79	24	345	0.64	4.1	ĸ	3
	1, 1	126	36	39	14	215	8.97	6.4	C	9
	$p_2$	201	65	69	22	357	50.0	0.4	ĸ	3
	ľ	125	77	42	11	222	0.94	5.2	ĸ	3
	t	429	133	135	30	727	54.5	2.9	C	3,6,8
$fg_2$	$w_{I}$	245	77	7.7	29	428	47.5	3.5	O	3,8
	e W	06	30	25	10	155	53.0	5.8	M	3
	də	91	27	33	5	156	41.0	9.9	M	3
	$fg_4$	247	19	20	71	357	12.0	1.8	C	3
	7,	187	71	59	28	345	53.0	3.9	M	3
	Ø	205	62	65	25	357	53.0	3.8	ĸ	3
	r	126	41	43	12	222	0.84	5.1	ĸ	3
	t	261	129	104	2	261	13.5	2.2	R	8,9
	$M_{\mathcal{I}}$	248	7.5	73	33	429	>55	3.8	82	3,8

continued ...

Summary of locus-to-locus linkage data in soybean (continued)

Cenes	v		- Phenotypic	Cl		Siim	% B	[II	Dhaca	Doforono
00110	0	AB	Ab	aB	ab		- 1	•	n 1	Ver er elice
$fg_A$	Ļ	188	73	58	26	345	52.0	3.9	M.	Э
$fg_{4}$	P2	208	61	62	26	357	55.0	3.7	×	3
$fg_4$	ľ	129	07	07	13	222	50.0	5.0	M	3
$fg_{4}$	t	111	47	63	0	221	0	0.0	M	3
$fg_4$	$M_{\mathcal{I}}$	206	62	70	17	335	53.0	4.1	O	3
$fr_1$	$e^{b}$	810	225	251	119	1405	42.5	1.8	C	28
$f_{\mathcal{L}_{\mathcal{I}}}$	иш	813	277	264	98	1440	50.1	2.0	C	11
$fr_1$	$t_{\mathcal{I}}$	846	292	290	111	1539	51.2	1.9	æ	2,27
t	+	27.5	7.0	σ	36	7,30	ι. ι.	o c	c	7.0
ת	$I_{\gamma n}$	7			0 7	t C	•	0.0	ر	/ 7
ŗ,	ms <sub>2</sub>	29	43	29	19	158	52.3	5.8	æ	17
ż	$w_I$	477	175	130	99	848	54.5	2.4	×	3,17
К,	ep	398	142	113	97	669	48.1	2.8	O	25
K 2	$fr_{j}$	607	131	116	43	669	48.0	2.8	O	25
k <sub>2</sub>	1,1	113	48	39	12	212	54.5	5.4	O	25
k <sub>2</sub>	qd	411	115	129	77	669	47.2	2.8	C	25
k2	t	374	125	119	39	657	8.67	2.9	M	2
k2	t	3186	1049	1006	356	5597	0.67	1.0	C	25
k <sub>2</sub>	94	497	147	141	48	833	52.0	2.5	R	25

Summary of locus-to-locus linkage data in soybean (continued)

		Д	- Phenotynic	120000						
Genes		AB	Ab	- 1	ab	Sum	% R	S.E.	Phase	Reference
1,1	$fg_1$	146	48	61	22	277	48.7	7.7	O	8
7 7	$fg_2$	151	77	59	23	277	45.9	4.3	O	∞
	$fg_3$	146	20	61	22	279	9.05	4.5	×	8
	k <sub>2</sub>	113	48	39	12	212	54.5	5.4	O	2
	ms <sub>2</sub>	207	71	80	28	386	49.7	3.8	O	8
	t t	161	36	61	21	279	0.44	4.2	O	8
	t	165	26	37	8	236	54.5	9.4	ĸ	2
	$M_{\mathcal{I}}$	356	117	146	77	663	51.2	3.0	O	8,17
	$ms_2$	72	30	12	7	121	55.0	7.9	В	19
	$ms_2$	124	40	32	22	218	39.7	4.5	O	17
$1f_1$	$w_{1}$	116	8 7	40	14	218	52.4	5.2	O	17
	$ms_2$	121	39	37	14	211	52.5	5.0	R	17
	$w_{1}$	120	40	40	11	211	47.3	5.3	ĸ	17
	$ms_2$	102	33	33	18	163	52.0	5.7	R	19
	$ms_2$	70	16	32	6	127	51.3	9.9	×	17
	$w_{\mathcal{I}}$	74	12	34	7	127	8.64	6.7	M	17

continued ...

Summary of locus-to-locus linkage data in soybean (continued)

Ð	Genes	AB	- Phenotypic Ab	classes	qe	Sum	% R	S. H.	Phase	Reference
IWI	csm	ms <sub>2</sub> 31		20	6	92	50.8	8.5	24	17
IWI	$M_{I}$	36	11	23	9	92	45.0	9.1	M	17
I	I									
ms <sub>3</sub>	$t_{\mathcal{I}}$	743	247	238	82	1310	50.5	2.8	М	27
$ms_3$	$M_{\mathcal{I}}$	170	55	61	16	302	47.2	7.7	W	27
п	ms <sub>2</sub>	164	99	47	16	303	6.44	9.4	Ж	17
п	$W_{\mathcal{I}}$	175	55	52	21	303	53.5	4.1	×	17
$p_1$	$fr_1$	291	91	102	32	516	50.0	3.0	C	14
$L_{d}$	1n	579	197	212	63	1053	П	2.4	C	15
$L_{\mathcal{A}}$	ms <sub>2</sub>	231	99	96	27	240	50.7	3.7	C	17
$D_{1}$	$M_{\mathcal{I}}$	223	74	100	23	420	55.4	3.9	C	17
$p_{J}$	y 9	785	270	291	87	1427	51.8	2.0	O	14,15
$p_2$	ln	213	53	65	25	356	0.44	3.7	C	4
P2	ms <sub>2</sub>	07	17	14	9	77	50.3	8.5	Я	17
p2	$W_{\mathcal{I}}$	253	75	79	97	433	>55	3.8	ĸ	3,17
qd	də	655	204	213	174	1146	>55	2.36	R	2,27
qd	$fr_{\mathcal{I}}$	1259	428	420	140	2247	49.7	1.5	R	2,27
qd	$t_1$	099	217	201	92	1154	87	2.2	C	2,27
qd	иш	1096	395	907	137	2037	50.8	1.4	O	11

continued ...

Summary of locus-to-locus linkage data in soybean (continued)

Genes		AB	henotypic Ab	classes — aB	ab	Sum	% R	S.E.	Phase	Reference
pc	ms <sub>2</sub>	160	56	37	16	269	52.5	7.7	M	17
pc	$I_{\mathcal{M}}$	160	99	43	10	269	44.5	6.4	œ	17
$pd_{J}$	ms <sub>2</sub>	215	69	56	33	373	14.8	3.5	Ö	17
$pd_1$	$I_{\mathcal{M}}$	227	57	69	20	373	47.8	3.8	ပ	17
$pd_2$	ms <sub>2</sub>	97	13	10	7	73	8.44	8.3	O	17
$pd_2$	$I_{\mathcal{M}}$	43	16	11	ю	73	9.45	9.2	O	17
$ps$ $ms_2$ 91 $2$	ms <sub>2</sub>	91	27	30	∞	156	51.4	6.1	C	17
sd	$\mathcal{I}_{\mathcal{M}}$	66	19	25	13	156	36.3	5.1	O	17
ľ.	ms <sub>2</sub>	80	35	36	6	160	42.5	7.9	ĸ	17
Z.	$I_{\mathcal{M}}$	241	55	73	24	383	53.5	5.7	ĸ	3,17
$rj_1$	£	756	271	317	54	1398	40.0	2.2	ĸ	15
$rj_1$	$fr_1$	292	114	76	32	532	48.2	3.3	M	14,15
$rj_J$	$fr_2$	259	29	97	28	451	51.2	3.0	M	15
$rj_1$	7	115	31	77	14	204	48.0	5.0	C	15
$rj_{\mathcal{I}}$	IE	108	36	30	12	186	52.6	5.0	×	15
$rj_1$	In	107	28	24	14	173	>55	5.0	ĸ	16
$rj_{1}$	d	115	33	34	6	191	51.0	5.0	C	16

Summary of locus-to-locus linkage data in soybean (continued)

			Phenotynic	200000			- 1			
Genes		- 1	Ab	- 1	ab	Sum	% B	S.E.	Phase	Reference
rjı	rps		45	74	16	364	0.64	4.0	C	15
$rj_{j}$	t		41	37	10	198	0.97	5.0	M	13
$rj_{j}$	W		80	72	22	396	47.7	3.8	M	13
$rj_1$	£ 6		133	128	50	702	51.8	2.8	ĸ	15
rjz	$fr_1$	300	115	116	31	670	55.5	3.3	O	15
$rj_2$	1,1		11	20	9	92	26.0	7.0	X	14,15
$rj_{\mathcal{A}}$	1,		34	27	5	198	45.0	0.9	R	14
rja	<sup>T</sup> d		251	248	59	1288	8.44	2.2	N	13,15,16
$rj_{4}$	99		37	27	13	196	0.44	5.0	Ö	13
rmd	$fg_1$		55	97	13	273	47.2	4.7	N	∞
rmd	$fg_2$		20	97	14	273	49.2	4.5	K	∞
rmd	$fg_3$		51	34	23	275	39.1	3.9	C	∞
rmd	1,1		09	39	19	274	53.2	7.7	M	∞
rmd	t		45	48	10	274	46.7	4.7	X	∞
rmd	t		16	17	9	107	7.77	8.9	C	7
rmd	$W_{\underline{I}}$		53	97	12	274	7.0	4.7	ĸ	∞

continued ...

Summary of locus-to-locus linkage data in soybean (continued)

Genes			Phenotypic	classes		Crim	0 %	0	1000	D & E
		AB	Ab	aB	ab	Dan.	- [	O.E.	riidse	Net er ence
rps	$fg_1$	160	56	77	14	274	48.4	9.4	ĸ	8
rps	$fg_2$	167	67	41	17	274	54.8	4.3	×	<b>∞</b>
rps	$fg_3$	161	99	77	14	275	51.2	9.4	O	8
rps	1,1	153	79	42	17	276	44.5	4.5	ĸ	8
rps	rmd	164	51	50	7	272	>55	4.8	U	∞
rps	rsv2	54	12	20	7	93	43.7	7.2	O	6
rps	t t	168	77	87	16	276	53.2	7.7	R	80
rps	$\nu_{J}$	169	48	07	17	274	>55	8.4	×	∞
Ŋ	"SW	147	67	87	10	254	56.4	5.0	O	17
Ŋ	T <sub>M</sub>	152	77	20	8	254	57.5	5.1	O	17
$st_2$	f.	713	461	229	166	1569	51.7	1.8	æ	1,21
t,	$df_5$	1530	142	156	375	2203	14.5	0.81	C	25
t	də	1230	379	395	121	2125	51.6	1.6	O	2,24
t	də	399	114	123	50	989	54.8	2.7	×	2,4
t	£	1619	554	514	183	2870	49.5	1.3	O	1,2,27
t	$fg_1$	157	79	50	7	278	>55	4.8	O	8
t	cam	333	192	120	57	702	52.8	0.9	O	11
t	ms <sub>2</sub>	80	35	36	6	160	42.5	7.9	ĸ	17
t	$ms_2$	246	92	89	34	077	46.2	3.4	C	17
t	949	472	147	166	48	833	48.8	2.6	R	27
ti	Le	59	17	15	5	96	52.0	7.4	×	22

Summary of locus-to-locus linkage data in soybean (continued)

Genes		AB Ph	Phenotypic classes Ab aB	classes —	ab	Sum	% R	S.	Phase	Reference
td	ms <sub>2</sub>	1	65	65	18	397	51.7	3.7	R	17
td	$I_{M}$	226	88	62	21	397	9.74	3.9	R	17
$\mathcal{I}_{M}$	$ms_1$	1268	557	583	09	2468	30.4	1.8	R	23
$\mathcal{I}_{\mathcal{M}}$	$ms_1$	451	91	87	100	729	27.9	2.0	C	23
$\mathcal{I}_{\mathcal{M}}$	$ms_2$	3574	1163	1034	372	6143	48.6	6.0	C	17
$I_{M}$	t	1792	580	587	234	3193	52.8	1.3	R	2,4,17
$\mathcal{I}_{M}$	t	171	67	36	16	272	45.0	4.3	C	∞
$W_{\mathcal{I}}$	WTM	333	9	7	107	450	2.2	0.5	C	5
$V_{A}$	t	72	21	23	10	126	55.5	6.2	R	e
$W_{oldsymbol{\mathcal{Q}}}$	WITH	71	22	25	∞	126	50.0	6.7	X	3
wm	$ms_2$	133	34	36	6	212	9.65	5.3	R	17,19
WTTI	$t_{\mathcal{I}}$	7.5	21	20	10	126	42.0	6.1	C	C)
7.2	<i>4</i> .	1053	627.	969	000	00%	0	c	٩	-
9	2 , ,	0061	470	070	7007	2403	7. V.	1.2	×	7 7
910	$W_{\underline{I}}$	3938	1329	1271	441	2269	49.5	0.8	O	27
912	$W_{\underline{I}}$	106	35	38	14	193	51.8	5.3	ĸ	27

Summary of locus-to-locus linkage data in soybean (continued)

				7						
9	200		Phenotypic	classes -		Sim	Q /⁄2	C	טיייטיים	J 0 C 0 C 0 C
	1100	AB	Ab	aB	ab	June		о. Б.	rnase	Kererence
420k2	$d\Theta$	458	154	174	62	878	50.8	2.5	R	26
420k2	$fg_1$	228	78	89	24	398	50.4	3.8	R	26
420k2	$t_1$	852	247	253	96	1448	46.2	2.0	O	26
y20k2	$M_{I}$	3124	975	766	334	5427	51.0	1.0	R	26
	a b	α	p	0	q	۵l	44	Sum		Reference
$Ap^{b}w_{I}$	$Ap^C_{M_{\underline{1}}}$	21	77	28	11	22	80	134		18
${\it Sp}_1^{\ a}{\it Dt}_1$	$Sp_1^{\ \ b}dt_1$	42	83	42	32	20	13	232		21
$Sp_1^{a}Ep$	$Sp_1^b ep$	70	91	77	24	18	15	232		21
$Sp_1^{a}Le$	$Sp_1^{\ \ b}$ le	20	67	23	11	6	∞	120		21
$Sp_1^{a_{W_1}}$	$Sp_1^{b_M}$	77	78	67	31	13	11	232		21
$Ti^{\mathcal{I}}_{\mathcal{D}}t_{\mathcal{I}}$	$Ti^2 dt_1$	34	83	20	24	24	17	272		21
$Ti^{}Ep_{1}$	$Ti^2 ep_1$	70	80	55	27	19	11	232		21
$Ti^{\mathcal{I}_W}$	$Ti^2_{W_I}$	42	82	53	25	21	6	232		21

 $^{\dagger}$  Values calculated by the method of Immer and Henderson (1943). Genetics 28:419-440.

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### 10) <sup>^</sup>Summary of trisomic linkage data in soybean //

Primary trisomics are useful for locating genes and linkage groups on specific chromosomes through the modification of genetic ratios by the extra chromosome. They also are useful in studying the phenotype and biochemical effects of individual chromosomes.

Three primary trisomics (Palmer, 1976) were used in attempts to locate mutants to one of these chromosomes. No distinct morphological differences exist among the three trisomics, or between them and their respective disomic sibs. Therefore, all parent plants of the trisomics and all  $F_1$  plants were checked for mitotic chromosome number (Palmer and Heer, 1973).

Trisomics A, B, and C were crossed to 29 mutants. Disomic and trisomic  $F_1$  plants were threshed individually. The following year, segregation ratios were determined. Many seedling traits were classified on sandbench-grown plants. Adult plant traits were classified on field-grown plants. We used the standard disomic  $F_2$  ratios, rather than theoretical ratios, as standards for comparison with the observed trisomic  $F_2$  ratios.

A total of 29 mutants, representing 9 of the 13 linkage groups in soybean were used; the data are summarized in Tables 1 and 2. In Table 1, the nine linkage groups represented among the 29 mutants tested are listed. Seven of the linkage groups have been tested against all three primary trisomics.

In Table 2, mutants are listed alphabetically, and segregation ratios between trisomic progenies are compared with disomic progenies. Only one mutant, a chimera that segregates as a single-gene recessive, has been assigned to a chromosome on the basis of trisomic inheritance tests. This is trisomic A.

Genetic studies using trisomic chromosomes in soybeans are underway, but it will be many years before all 20 primary trisomics are identified.

Table 1. Linkage groups, mutants tested, and trisomics tested in soybean trisomic inheritance studies

Linkage group	Mutants	Trisomics tested
1	df <sub>5</sub> , t, y <sub>1</sub> , y <sub>2</sub>	А, В, С
3	g	В
4	ln	A, B, C
5	$dt_{1}$	A, B, C
6	y <sub>11</sub>	А, В, С
7	y 13	Α, Β
8	w <sub>1</sub> , ms <sub>1</sub>	А, В, С
11	rj <sub>1</sub> f	А, В, С
13	fr <sub>1</sub>	А, В, С
13	ep	А, В

Table 2. Chi-square tests of  $F_2$  trisomic segregation ratios compared with observed  $F_2$  disomic segregation ratios for various soybean mutants

Mutant	Trisomic	A	В	С	D	chi <sup>2</sup>	Ref.
chimera	А	3.69	8439	18.39	3160	492.18**	7
Df5 df5	A	2.85	354	3.69	455	5.12	10
Df5 df5	B	2.69	443	3.35	448	3.83	
Df5 df5	C	3.29	146	2.82	443	2.03	
Dt1 dt1	A	3.42	159	3.21	736	0.53	9
Dt1 dt1	B	3.20	189	3.33	740	0.20	
Dt1 dt1	C	2.96	182	3.23	575	0.79	
Ep ep	A	3.28	278	3.23	127	0.00	9
Ep ep	B	2.66	326	3.07	395	1.50	
F f	A	3.28	672	3.23	1095	0.04	1
F f	B	3.19	742	2.94	922	1.16	
F f	C	3.13	409	2.96	995	0.58	
Fg1 fg1	A	3.35	100	2.45	100	2.03	9
Fg1 fg1	B	2.57	100	3.55	100	1.80	
Fr1 fr1	A	2.88	2659	3.09	1467	1.34	2
Fr1 fr1	B	2.90	1343	3.15	1358	1.69	
Fr1 fr1	C	3.07	1480	3.25	1101	0.64	
Fr2 fr2	A	3.45	1741	3.25	999	0.64	2
Fr2 fr2	B	3.16	1656	3.30	331	0.11	
Fr2 fr2	C	2.99	2058	2.94	1191	0.06	
Fs1Fs2 fs1fs Fs1Fs2 fs1fs Fs1Fs2 fs1fs	s2 B	13.40 13.40 14.30	1945 562 768	15.60 16.20 13.40	1148 187 722	1.50 0.36 0.19	13
G1 g1	В	2.85	909	3.28	775	2.74	8
Ln ln	A	3.46	125	3.17	429	0.59	8
Ln ln	B	3.02	233	3.22	435	0.32	
Ln ln	C	2.98	382	2.83	134	0.06	
Ms1 ms1	A	2.89	1293	2.72	387	0.28	9
Ms1 ms1	B	2.86	521	2.43	474	2.60	
Ms1 ms1	C	2.48	386	3.02	380	2.76	

A = disomic ratio; B = number of disomic plants; C = trisomic ratio; D = number of trisomic plants

Mutant	Trisomic	А	В	С	В	chi <sup>2</sup>	Ref.
Ms2 ms2	A	2.92	501	2.90	555	0.00	5
Ms2 ms2	B	3.03	483	2.88	751	0.36	
Ms2 ms2	C	2.83	340	3.03	801	0.69	
Ms3 ms3	A	2.48	310	2.85	520	1.93	13
Ms3 ms3	B	2.94	627	3.41	785	3.03	
Ms3 ms3	C	2.88	279	3.09	527	0.48	
Ms4 ms4	A	2.90	2707	2.82	2228	0.33	3
Ms4 ms4	B	3.04	3385	2.84	1933	1.72	
Ms4 ms4	C	3.05	1073	2.98	972	0.09	
Pb pb	A	2.92	2659	2.73	1467	1.30	2
Pb pb	B	2.87	1343	3.20	1358	2.92	
Pb pb	C	2.97	1480	3.00	1101	0.02	
Rj1 rj1	A	2.86	320	2.76	809	0.19	9
Rj1 rj1	B	2.98	803	3.13	818	0.36	
Rj1 rj1	C	2.98	789	2.95	1119	0.02	
Rj4 rj4 Rj4 rj4	A B	3.17 3.15	313 514	3.25 3.26	1100 1128	0.12	4
Rxp rxp	A	3.07	1071	2.88	946	0.73	6
Rxp rxp	B	2.92	762	2.82	680	0.16	
T t	A	3.20	290	3.22	489	0.00	9
T t	B	2.96	1102	3.03	826	0.08	
T t	C	3.31	851	3.03	901	1.31	
W1 w1	A	3.00	3808	2.93	2507	0.26	9
W1 w1	B	2.92	2527	3.08	2139	1.12	
W1 w1	C	2.86	1879	3.04	1290	0.89	
Y9 y9	A	2.99	1852	3.02	1257	0.02	2
Y9 y9	B	2.86	2865	2.81	2127	0.12	
Y9 y9	C	3.16	2328	3.01	1813	0.80	
Y10 y10	A	3.32	2301	3.01	1440	2.59	8
Y10 y10	B	3.10	2228	3.14	1588	0.04	
Y10 y10	C	3.06	1278	3.00	776	0.05	
Y11 y11	A	2.91	824	2.75	1283	0.80	8
Y11 y11	B	3.20	1502	3.17	921	0.01	
Y11 y11	C	2.74	1550	3.64	881	12.09*	

A = disomic ratio; B = number of disomic plants; C = trisomic ratio; D = number of trisomic plants

Mutant	Trisomic	A	В	С	В	chi <sup>2</sup>	Ref.
Y12 y12	A	3.39	411	3.46	183	0.01	9
Y12 y12	B	3.01	333	2.74	161	0.28	
Y12 y12	C	3.35	209	2.91	227	0.85	
Y13 y13	A	3.45	365	3.68	604	0.42	8
Y13 y13	B	2.77	279	3.23	1965	8.39*	
Y18 y18	A	3.22	308	3.41	474	0.27	8
Y18 y18	B	3.99	2573	3.67	1490	1.75	
Y19 y19	A	3.86	1243	3.26	711	3.65	9
Y19 y19	C	3.56	1501	3.08	528	2.05	
Y20K2 y20k2 Y20K2 y20k2 Y20K2 y20k2	2 B	3.51 3.11 3.49	415 1102 763	3.28 2.97 3.54	394 826 618	0.32 0.33 0.02	11

A = disomic ratio; B = number of disomic plants; C = trisomic ratio; D = number of trisomic plants

<sup>\*\* =</sup> Statistically significant (P = .01)
\* = Statistically significant (P = .05); however, based on the
 observed ratios, these are not believed to be biologically
 significant. These mutants are being tested further.

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1) A method for isolating poly(A)-containing RNA from soybean suspension culture cells for cDNA synthesis and cloning.

Our laboratories are interested in using recombinant DNA techniques to study the genetics of amino acid metabolism in soybeans. An especially useful technique used to study gene regulation is the *in vitro* synthesis of DNA complementary to mRNA; this cDNA can be used directly as a hybridization probe, or cloned and amplified in a suitable host. The protocol described here was developed for isolating total cellular poly(A)-containing RNA from soybean suspension culture cells of sufficient purity for use as a template for cDNA synthesis. In addition, preliminary experiments were performed to determine the feasibility of constructing a cDNA clone bank in the *E. coli* cloning vector M13mp7.

### Materials and methods

Preparation of plant material. *G. max* root cell cultures SBe4, GMW, and M24 were maintained as exponentially growing suspension cultures in B5 medium (Gamborg et al., 1968). Two-day-old cultures were filtered through a  $10\text{--}40\,\mu\text{m}$  sintered glass filter and washed with three volumes of ice cold saline. The cell mass was then frozen at -70°C, lyophilized, and stored in airtight containers at 4°C.

RNA extraction. All buffers and glassware were either autoclaved or treated with 0.1% diethylpyrocarbonate.

Total cellular RNA was extracted from lyophilized soybean cells as suggested by Murray and Thompson (1980), with the incorporation of 15mM Na iodoacetate into all solutions. The RNA, pelleted during CsCl/ethidium bromide ultracentrifugation, was dissolved in low-salt buffer plus 15mM Na iodoacetate and extracted several times with saturated 2-propanol to remove ethidium bromide (Maniatis et al., 1982). RNase-free DNase I was added to a concentration of 5  $\mu g/ml$ , and incubated for 10 minutes at room temperature. The RNA was then ethanol precipitated, pelleted, and redissolved in oligo(dT)-cellulose application buffer.

Poly(A)-containing RNA isolation. Poly(A)-containing RNA was first purified from the total cellular RNA by oligo(dT)-cellulose chromatography as described by Aviv and Leder (1972), using buffers suggested by the supplier of the column matrix (Collaborative Research, Inc.). The poly(A)-containing RNA was further purified by pooling the A260 elution peak, adding phosphate buffer (pH 6.8) to a concentration of 10mM, and applying to a hydroxyapatite column. After extensive washing with 10mM phosphate buffer (pH 6.8) the RNA was eluted with 0.4M phosphate buffer (pH 6.8) (Beachy et al., 1980). The fractions containing the A260 elution peak were again pooled, and added to an equal volume of 2X oligo(dT)-cellulose application buffer, followed by a second round of purification on oligo(dT)-cellulose. The purified poly(A)-containing RNA was then precipitated with ethanol, pelleted, dissolved in distilled and deionized water to a concentration of 1  $\mu$ g/ $\mu$ 1, and stored at -70°C.

 $3^2P\text{-cDNA}$  synthesis. Single-stranded  $3^2P\text{-cDNA}$  was synthesized as described by Wickens et al. (1978), using 5  $\mu\text{Ci}$  of  $\alpha^{3\,2}P\text{-dCTP}$ , 5  $\mu\text{g}$  of poly(A)-containing RNA, and 100 units of reverse transcriptase. A 2.5  $\mu\text{l}$  aliquot was removed and separated by gelfiltration on a 0.5 x 35 cm G50(50-150) sephadex column. Fractions of approximately 0.5 ml were collected and counted by scintillation spectrophotometry.

Second strand synthesis. Double-stranded cDNA was synthesized from single-stranded cDNA as described by Wickens et al. (1978), using 5  $\mu$ Ci of 8-3H-dATP, and 50 units of DNA polymerase I. After extraction with chloroform, the entire sample was separated by gel filtration on a 0.5 x 35 cm G50(50-150) sephadex column, collecting 0.5 ml fractions. A 10  $\mu$ l aliquot of each fraction was removed and counted by scintillation spectrophotometry. The fractions containing the cDNA peak were precipitated with 2-propanol (Maniatis et al., 1982).

S1 nuclease treatment. Hairpins and residual single-stranded cDNA were removed in a 500  $\mu I$  reaction mixture with S1 nuclease as described by Wickens et al. (1978). Resistance to S1 nuclease was determined by gel filtration of 25  $\mu I$  pre- and post-S1 nuclease aliquots as described for assaying  $^{32}P$ -cDNA synthesis. S1 nuclease treated double-stranded cDNA was then precipitated with ethanol.

Cloning. In brief, S1 nuclease treated double-stranded cDNA was endrepaired and blunt-end ligated directly into  $\underline{\text{Hinc-II}}$  cleaved M13mp7 RF DNA. The ligation reaction was used to transfect competent  $E.\ coli$  JM103 cultures, which were then plated on media containing IPTG and Xgal. Presumptive clones (colorless plaques) were isolated and confirmed by electrophoresis of native and restriction enzyme digested RF DNAs. A restriction map of one of the clones (wp02) was constructed from restriction analysis.

#### Results

Poly(A)-containing RNA isolation. Total cellular and poly(A)-containing RNA preparations were evaluated by staining urea-agarose gels with acridine orange (Locker, 1979). Total RNA preparations contained high molecular weight single-stranded (red-fluorescing) nucleic acid with distinct bands corresponding to approximately 4-6s, 18s, and 30s RNA. No double-stranded (green fluorescing) or RNase-resistant material was visible.

cDNA synthesis. The synthesis of cDNA was followed by the incorporation of  $^{32}\mathrm{P}$  and  $^{3}\mathrm{H}$  counts into the sephadex G50 excluded fraction. The normal yield of single-stranded cDNA was about 100 ng, although yields as high as 750 ng have been obtained. Yields of double-stranded cDNA were usually 200-500 ng.

Sl nuclease treatment. The resistance of the double-stranded cDNA to Sl nuclease was determined by the release of  $^{32}P$  and  $^{3}H$  counts from the sephadex G50 excluded fraction into the included fraction. By counting the  $^{32}P$  and  $^{3}H$  channels separately, resistance of  $^{3}H$ -cDNA (double-stranded) as compared with  $^{32}P$ -cDNA (total) was determined;  $^{32}P$ -cDNA was 75% resistant to Sl nuclease, while there was no detectable sensitivity of  $^{3}H$ -cDNA to Sl nuclease.

 $\overline{\text{Cloning}}$ . A total of 5 confirmed clones were isolated from two separate cDNA syntheses. Restriction analysis showed that the clones were all nearly identical, containing insertions with the same restriction pattern inserted

in the same orientation. There appear to be slight variations (up to about 10 base pairs) in the length of cDNA inserted; the average is 350 base pairs. The restriction map of wp02 shows that the 350 base pair cDNA insert has l  $\underline{\text{Taq}}$  I site, 2  $\underline{\text{Sau}}$  3A I sites, and 3  $\underline{\text{Hae}}$  III sites. There are no  $\underline{\text{Hinc}}$  II,  $\underline{\text{Pst}}$  I,  $\underline{\text{Sal}}$  I, Kpn I, Bam HI, Eco RI, Hind III or Hpa I sites within the cDNA insertion.

### Conclusion

A method for isolating highly purified poly(A)-containing RNA from soybean suspension culture cells has been formulated. The poly(A)-containing RNA is free of apparent contamination with DNA, rRNA, and tRNA, and is suitable for use as a template for double-stranded cDNA synthesis. Although cloning this cDNA has met with limited success, presumably because (as was recently shown by J. Messing, personal communication)  $E.\ coli$  JM103 is restriction plus rather than restriction minus, as was previously thought. The recent development of M13 cloning vector hosts that lack restriction activity should eliminate this barrier, making the construction of a soybean cDNA gene bank in M13 a feasible goal.

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1) Electrophoretic classification of selected G. max plant introductions and named cultivars in the late maturity groups

Over the last few years, we have been classifying various G. max and G. soja lines based on their electrophoretic zymogram patterns. In the 1982 Soybean Genetics Newsletter (Gorman et al., 1982), we published a table compiling electrophoretic profiles for most of the named soybean cultivars in the early maturity groups (000-IV). Listed on the table below is a similar compilation of electrophoretic profiles (zymogram types for 12 enzyme systems) for 60 G. max PIs (10 originating from Northeast China, 10 from Central China, 20 from Korea, and 20 from Japan) as well as 90 named cultivars in the late maturity groups (V-VIII). Descriptions of these zymogram types and/or information on their inheritance patterns have been published previously (Gorman, 1983; Gorman et al., 1983; Kiang and Gorman, 1983; Gorman et al., 1982; Kiang, 1981; Gorman and Kiang, 1978). More than one variable locus has been identified in either G. max or G. soja for several of the enymzes (ADH, Am, Dia, LAP, IDH, PGD, PGM and TO) and all of the zymograms, except MPI, represent the products of more than one enzymatic locus. Thus, most of the zymogram types represent multiple loci phenotypes. Each number in the body of the table represents the zymogram type observed for the particular enzyme (columns) and cultivar or PI (rows). The enzyme abbreviations used were: ADH for alcohol dehydrogenase, Am for amylase, AP for acid phosphatase, Dia for diaphorase, GPD for glucose-6-phosphate dehydrogenase, IDH for isocitrate dehydrogenase, LAP for leucine amino peptidase, MPI for mannose-6-phosphate isomerase, PGD for phosphogluconate dehydrogenase, PGI for phosphoglucose isomerase, PGM for phosphoglucomutase and TO for tetrazolium oxidase. The initial electrophoretic screening consisted of an examination of five seeds (electrophoresed in four different gels) from each cultivar or PI, tested for all 12 enzymes. If all of the enzymes were not satisfactorily resolved, or when unusual results (i.e., mixed zymogram types) were obtained, additional seeds were tested in subsequent electrophoretic runs. Thus, each number in the body of the table represents the observations made on a minimum of five seeds. When two or more numbers are listed, the cultivar or PI had a mixture of these zymogram types. Cultivars and PIs were considered to have a mixed zymogram only when seeds were classified into two or more zymogram types on repeated electrophoretic runs. only one or two seeds from a single electrophoretic run showed a different zymogram from the majority of seeds for that line, these seeds were considered atypical and the cultivar or PI was not considered mixed. It was felt that one or two unreplicated seeds with abnormal zymograms were more likely the result of scoring or seed handling mistakes by us, rather than line impurities. PI seeds were obtained from Dr. R. L. Bernard (USDA-ARS at the University of Illinois), while seeds from the late maturity cultivars were obtained from Dr. T. C. Kilen (USDA-ARS at Stoneville, MS).

### Zymogram Types

PI or Cultivar	Enzymes											
	ADH	Am	то	AP	LAP	PGD	GPD	PGM	Dia	MPI	IDH	PGI
Northeast China												
103414	2	1	1	2	1	1	1	2	1	2	6	1
103415	1	2	1	3	1	2	1	2	4	1	3	1
103419	1	1	1	1	1	1,2	2	2	1	1	7	1
135589	1	1	1	3	1	2	1	2	2	2	2	1
135590	1	1	1	2	1	2	1	2	2	2	1	1
232987	1	1	1	1		2	1	2	2	2	1	1
232988	1	1	1	3		2	1	2	2	2	1	1
232989	1	1	1	3		2	1	2	2	2	1	1
232990	1	1	1	3		2	1	2	2	2	1	1
232991	1	1	1	3		1	1	2	2	2	2	1
Central China												
103080	1	2	1	3		1	1	1	1	2	1	1
103088	1	1	1	3		1	1	2	2	2	1	1
103091	1	2	1	2	1	1	1	1	1	2	1	1
123577	1	1	1	2	1	1	2	2	2	1	3	1
158765	1	1	1	2	1	1	1	1	3	3	3	1
253650A	1	1	1	2	1	1	2	1	2	1	7	1
253650B	1	1	1	1	1	1	1	2	4	2	5	1
253651A	1	1	1	2	1	1		1	1	2	3	1
253651B	1	2	1	1	1	1	2	1	2.	2	7	1
2536 <b>5</b> 3A	1	1	1	2	1	1	1	2	1	2	3	1
Korea												
157395	1,3	1	1	2	1	1	2	2	2	2	7	1
157396	ĺ	1	1	2	1	1	2	1	2	2	7	1
157396	1	1	1	2	1	1	1	2	2	2	7	1
157398	3	1	1	3	1	1,2	1	1	2	2	7	1
157401	1	1	1	1	1	1	2	2	1	2	3	3
157402	1	1	1	2	1	1	1	2	2	2	7	1
157404	1	1	1	2	1	2	2	2	2	2	7	1
157405	1	2	1	2	1	1,2	1	2	2	2	7	1
157408	1	1	1	2	1	1	2	1	2	2	7	1
157409	1	1	1	2	1	2	1	1	2	5	7	1
157410	1	2	1	1	1	1	1	1	1	2	7	1
157414	3	1	1	2	1	1	2	1	1	2	7	1
157416	1	1	1	2	1	1	2	2	1	2	3	1
157417	1	1	1	2	1	2	1	2	1	1	7	1
157419	1	1	1	2	1	2	1	1	1	1	7	1

#### Zymogram Types

						En	711m o c					
PI or Cultivar	ADH	Am	ТО	AP	LAP	PGD	zymes GPD	PGM	Dia	MPI	IDH	PGI
157421	1	2	1	3	1	1	1	2	2	2	7	1
157421	1	2	1	2	1	1	1	2	1	2	7	1
157428	1	2	1	2	1	1	2	2	1	2	7	1
157429	1	2	1	1	1	1	2	1	2	3	7	1
157431	1	1	1	2	1	1	2	1	2	2	7	1
137431	_	_	_	2	1	1	2	1	2	_	,	1
Japan												
124871	1	2	1	3	1	2	1	1	1	1	7	1
181531	1	1	1	2	1	2	2	2	1	5	7	1
181532	1	1	1	2	1	2		2	1	5	7	1
181533	1	1	1	2	1	1	2	2	4	2	3	1
181534	1	1	1	2		1	1	2	1	1	7	1
181535	3	1	1	2	1	1	1	2	2	2	6	1
181536	1	1	1	3	1	2	1	1	2	2	7	1
181537	1	1	1	2	1	1		1	2	2	7	1
181538		1	1	2	1	1	2	2	4	1	3	1
181539		1	1	2	1	1	2	1	4	2	3	1
181540	1	1	1	2	1	2		1	2	5	7	1
181541	1	1	1	2	1	2	1	1	2	5	7	1
181542	-	1	1	2	1	2	1	1	8	5	7	1
181548		1	1	2	1	2		2	2	1	7	1
181549		1	1	2	1	2		1	1	5	5	1
101349		1	1	2	1	2		1	1	)	5	1
181550	3	1	1	2	1	2	2	1	2	1	8	1
181551	1	1	1	2	1	1	2	1	2	2	5	1
181552	1	1	1	2	1	2	2	2	1	1	7	1
181553	1	1	1	2	1	1	2	1	2	5	7	1
181554	1	1	1	2	1	1	2	1	2	5	7	1
							_	_	_		•	-
Southern Maturity												
Acadian	1	1	1	2	1	1	2	2	2	3	3	1
Arisoy	1	1	1	2	1	1	2	2	2	3	3	1
Arksoy	1	1	1	2	1	1	1	1	2	2	3	1
Armredo	1	1	1	2	1	1	1	2	3	2	3	
Avoyells	1	1	1	2	1	1	2	2	1	2	5	1
Barchet	1	1	1	2	1	1	2	2	1	2	3	1
Bedford	1	1	1	2	1	1	2	1	1	2	5	1
Biloxi	1	1	1	2	1	1	1	2	1	2	3	1
Bosier	1	1	1	2	1	_	_	1	1	2	5	1
Bragg	1	1	1	2	1	1	2	1	1	2	5	1
51466	_	_	1	2	1	_	_	1	1	2	)	1
Centennial	1	1	1	2	1	1	2	1	2	2	5	1
Charlee	1	1	1	2	1	1	1	1	1	3	1	1
Cherokee	1	1	1	2	1	1	2	2	1	3	3	1
Creole	1	1	1	2	1	1	1	2	2	3	3	1
Clemson	1	1	1	2	1	1	1	2	2	1	1,3	1

Zymogram Types

				2) mo	514111 1							
or Cultivar	ADH	Am	ТО	AP	LAP	— Enz PGD	gPD	PGM	Dia	MPI	IDH	PG
Cobb	2	1	1	2	1	1	1	1	2	3	5	1
Coke Stewart	1	1	1	2	1	1	2	2	1	1	1	1
Columbus	1	1	1	2	1	1	1	2	1	2	5	1
Dare	1	1	1	2	1	1	1,2	1	2	2	7	1
Dortschoy	1	1	1	2	1	1	1,2	2	1,2	2	5,7	1
Davis	3	1	1	2	1	1	1	1	2	2	7	1
Delsta	1	1	1	2	1	2	2	2	1	1	2	1
Delsoy	2	1	1	3	1	1	1	2	2	1	7	1
Dixie	1	1	1	1	1	1	1	2	2	2	3	
Dyer	1	1	1	2	1	1	2	1	1,2	2	7	1
Easycook	3	1	1	1	1	1	1	1	1	2	3	1
Essex	3	1	1	2	1	1	2	1	1	2	5,7	_
	1	1		2			2			2		1
Forrest	_	_	1		1	1		1	1		5	1
Gaton	1	1	1	2	1	1	1	2	1	3	3	1
Georgian	1	1	1	2	1	1	1	2	2	2	3	1
Hampton 266	1	1	1	2	1	2	2	1	1	1	5	1
Haberlandt	1	1	1	3	1	1	1	1	2	2	7	1
Hardee	3	1	1	2	1	1	1	1	2	2	1	1
Harvell	1	1	1	2	1	2	1	2	7	2	7	1
Hayseed	1	1	1	2	1	1	1	1	2	2	1	1
Hinn	1	1	1	2	1	1	1	2	1	2	1	1
Hollybrook	1	1	1	2	1	2	1	1	2	2	7	1
Hood	1	1	1	2	1	1	1	2	2	2	7	1
Hutton	1	1	1	2	1	1	2	1	1	2	7	1
Imp'd. Pelica		1	1	1	1	1	1	2	1	3	3	1
T 1	2	1	1	2	1	2	1	2	1	2	5	1
Jackson	2	1	1	2	1	2	1	2	1	3	5	1
Jew 45	1	1	1	2	1	2	1	2	1	1	5	1
Jupiter	1	1	1	2	1	1	2	1	1	2	7	1
Kino	1	1	1	2	1	1	1	1	1	2	7	1
La Green	1,2	1	1	2	1	1	2	2	1,2	1	3	1
Laredo	1	1	1	2	1	1	1	2	4	2	1	1
Lee	1	1	1	2	1	1	2	1	2	2	5	1
Lee 68	1	1	1	2	1	1	1	1	1	2	1,5	1
Lee 74	1	1	1	2	1	1	1,2	1	1,2	2	1,5	1
Luthy	1	1	1	3	1	1	2	1	8	1	7	1
Mack	1	1	1	2	1	1	1	1	2	2	5	1
Magnolia	1	1	1	2	1	1	1	1	2	2	3	1
Majors	1	1	1	2	1	1	1	2	1	1	3	1
Mamloxi	1	1	1	2	1	1	2	2	1	3	2	1
Mammoth Yello	-	1	1	2	1	1	2	3	1	2	1,3	1
Mamredo	1	1	1	2	1	1	1	2	2	2	5	
		T	T									1
	_	1	1	2	7	7	')	')		٠,		
Manotan 6640	1	1	1	2	1	2	2	2	1	3	2	1
Manotan 6640 Missoy	1	1	1	2	1	1	1	1	1	3	1	1
Manotan 6640	1	_										

Zymogram Types

PI or Cultivar ADH Am TO <sup>a</sup> AP LAP <sup>b</sup> PGD GPD PGM Dia MPI IDH  Nansemond 1 1 1 2 1 2 1 2 7 2 7  Nela 3 1 1 1 1 2 1 2 1 2 2 2 1 8  Old Dominion 1 1 1 1 2 1 1 1 2 2 2 2 2 1  Palmetto 1 1 1 2 1 1 1 2 1 1 2 1 3 1  Pickett 1 1,2 2 1 1 2 1 2 5	PGI 1 1
Nansemond       1       1       1       2       1       2       1       2       7       2       7         Nela       3       1       1       1       1       2       1       2       1       2       2       1       8         Old Dominion       1       1       1       2       1       1       1       2       2       2       1         Palmetto       1       1       1       2       1       1       1       2       1       3       1	1
Nansemond       1       1       1       2       1       2       1       2       7       2       7         Nela       3       1       1       1       1       2       1       2       1       2       2       1       8         Old Dominion       1       1       1       2       1       1       1       2       2       2       1         Palmetto       1       1       1       2       1       1       1       2       1       3       1	
Nela     3     1     1     1     1     2     1     2     2     1     8       Old Dominion     1     1     1     2     1     1     1     2     2     2     1       Palmetto     1     1     1     2     1     1     1     2     1     3     1	
Old Dominion     1     1     1     2     1     1     1     2     2     2     1       Palmetto     1     1     1     2     1     1     1     2     1     3     1	1
Palmetto 1 1 1 2 1 1 1 2 1 3 1	
Palmetto 1 1 1 2 1 1 1 2 1 3 1	
	1
	1
	-
Pickett 71	1
Pine Delta	1
	1
Perfection 1 1 1 2 1 1 1 2 2 3	1
Pluto 1 1 1 2 1 1 1 2 1 2 1	1
Pochahontas 1 1 1 2 1 1 1 1 5	1
Ralsoy 1 1 1 2 1 1 1 2 2 3	1
Roanoke 2 1 1 2 1 2 1 2 5	1
Rokusun 1 1 1 2 1 2 1 1 1 1 5,6	1
Rose Non-Pop 1 2 1 3 1 1 2 1 2 2 7	1
S-100 2 1 1 2 1 1 2 1 1 2 5	1
Seminole 1 1 1 2 1 1 1 2 1,2 2 3	1
Semmes 1 1 1 2 1 2 2 1 1 2 7	1
Tanner 1 1 1 2 1 1 2 1 3 3	1
Tarheel Black 1 1 1 2 1 1 2 1 2 3	1
Tenn Non-Pop 2 1 1 2 1 2 1 5	1
Tokyo 1 1 1 2 1 2 1 2 2 7	1
man 3	
Tracy and	
Tracy-M 1 1 1 2 1 2 2 1 2 2 7	1
Volstate 3 1 1 2 1 2 1 1 2 5	1
White Biloxi 1 1 1 2 1 1 2 2 1 3 3	1
Wood Yellow 1 1 1 2 1 1,2 1 2 1 2 6	1
Yelnanda 1 1 1 2 1 1 1 2 1 1 4	1
Yelredo 1 1 1 2 1 1 1 2 1 1 4	1
York 1 1 1 2 1 1 1 2 2 5	1
Total number of	
seeds scored: 847 750 849 797 788 1033 810 788 908 700 1113	750
Total number of	
mixed lines: 2 0 1 0 0 3 3 0 5 0 7	0
Total number of	
Total number of atypical seeds: 2 0 0 2 0 6 2 2 3 2 8	0

<sup>&</sup>lt;sup>a</sup>Only the variable locus To<sub>4</sub> was scored in this table (Type-1 vs. Type-2 TO zymograms), an additional variant TO zymogram (Type-3) was not tested for.

 $<sup>^{\</sup>rm b}{\rm Only}$  the variable locus  ${\rm Lap}_1$  was scored in this table (zymogram types 1, 2 or 3), an additional LAP variable locus (Lap\_2) was not scored for.

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## 2) Linkage of electrophoretic loci/

In our studies of the inheritance of various electrophoretic variants, we have examined F<sub>2</sub> segregation data from many crosses (see Gorman, 1983, for a listing). Many of these crosses were segregating for multiple loci, allowing linkage data to be collected. Table 1 represents a summary of the linkage patterns we have observed between the listed electrophoretic loci (see Gorman and Kiang, 1978; Kiang, 1981; Gorman et al., 1983, concerning the establishment of these loci). Only the locus pairs for which we had data from a minimum of 95  $F_2$  seeds were included in Table 1, while just those pairs that showed an independent segregation pattern based on greater than 300  $F_2$  seeds, or a conclusive linkage pattern, were listed without question marks. The question marks were used to indicate insufficient data to firmly establish a linked pair or to detect the possibility of weak linkage. The product method (Immer and Henderson, 1943) was used to calculate F2 recombination percentages and standard errors. To facilitate the use of the product method, those loci showing a codominant segregation pattern had their heterozygous class bulked with one of their homozygous classes. Sixty-six gene pairs had F2 segregation ratios consistent with independent assortment, but in 45 of those pairs the number of seeds tested was too small to be able to eliminate the possibility of weak linkage. The segregation data and appropriate  $\chi^2$  values for those 7 gene pairs found to have a recombination fraction 2 or more standard errors below 0.5 are listed on Table 2. Three of these gene pairs, Adh, with Adh, (Gorman and Kiang, 1978), Ap with Lap, and Pgd with Pgi, showed conclusive linkage results. The establishment of possible linkage of  $Am_3$  with  $Lap_1$  and of  $Idh_1$ with Lap<sub>1</sub> was clouded by the significant deviation (from 3:1) observed in their single-locus ratios. The data for the Ap with  $Idh_1$  and Ap with  $Idh_3$  gene pairs are consistent with weak linkages, but the number of  $F_2$  seeds tested was too

small to detect a significant deviation from independence with a  $\chi^2$  test. However, since the Ap and  $Lap_1$  loci are clearly linked and since both the  $Idh_1$  with  $Lap_1$  and Ap with  $Idh_1$  gene pairs gave indications of linkage, it seems likely that Ap,  $Lap_1$  and  $Idh_1$  belong in the same linkage group. Hildebrand et al. (1980) reported that the Ti and Ap loci were linked, belonging to linkage group 9. Therefore,  $Lap_1$  can be placed in linkage group 9, with  $Idh_1$  likely also belonging to this group. Since  $Am_3$  showed independent segregation with Ap,  $Idh_1$ , and Ti (Gorman, 1983; Hildebrand et al., 1980; Orf and Hymowitz, 1977), its membership in this group is questionable. The linkage groups to which the  $Adh_1$  with  $Adh_2$  and the Pgd with Pgi gene pairs belong is not known.

Table 1. Linkage relationships between electrophoretic loci

								L	oci						
	$Adh_4$	$Am_3$	Ap	Di <sub>1</sub>	Di <sub>2</sub>	Di <sub>3</sub>	Gpd	$^{Idh}_{1}$	$Idh_2$	$Idh_3$	Lap <sub>1</sub>	Mpi	Pgd	Pgi	$Pgm_1$
$Adh_1$	L	ı? <sup>a</sup>		I?			I?	I?	I?		I?	L?			
$Am_3$			I?	I	I?		I?	I	I?	I	?	I?			I
Ap				I?				L?		L?	L		I?	I?	I?
Di 1					I?	I?	I	I?	I	I?	I	I?	I?		I?
Di <sub>2</sub>						I?	I?	I?	I?	I	I?	I?	I?		I?
Di <sub>3</sub>										I?		I?	I?		
Gpd								I?	I	I	I?	I	I		I
$Idh_1$									I	1?	L?	I?	I?		1?
Idh <sub>2</sub>										I	I	I?			I
$Idh_3$											I?	I?	I?		I
Lap <sub>1</sub>												ī?			I
Mpi													I		I
Pgd														L	I
Pgi															

 $<sup>^{</sup>a}$ Only gene pairs with 95 or more segregating  $\mathrm{F}_{2}$  seeds were included in the table, while only gene pairs with greater than 300 segregating  $\mathrm{F}_{2}$  seeds or conclusive linkage data were typed without a question mark. I stands for independent assortment, while L stands for linked loci.

2 or more S.E. below 0.5 Examination of the gene pairs with a recombination fraction of Table 2.

Gene	Pair B	Phase	A	Phenotypic B	ic class C	Ω	$\chi^2(3:1)$ Locus A	$\chi^2(3:1)$ Locus B	$\chi^2$ (9:3:3:1) Product/Method Loci A & B %R S.E.	Product , %R	:/Method S.E.	Conclusion
Adhı	: Adh <sub>4</sub>	O	315	3	9	122	3.25	2.17	484.0**	2	1%	Linked
Am3	: Lap <sub>1</sub>	R	562	192	168	34	7.6**	0.95	13.81**	43	3%	Questionable
Ap	$: Idh_1$	K 0	65	37	32	3 7	0.49	2.00	7.10*	36	%9	Possible, but inconclusive
Ap	$: Idh_3$	S C	29	7	13	1 6	60.0	3.60	3.14	38	%9	Possible, but inconclusive
Ap	: Lap <sub>1</sub>	æ	452	202	214	12	0.21	0.21	60.21**	23	3%	Linked
$Idh_1$ :	: Lap <sub>1</sub>	C	185	53	70	42	9.17**	0.85	21.90**	0 7	% 7	Possible, but inconclusive
Pgd	: Pgi	O	112	14	19	27	0.28	0.12	42.80**	21	%7	Linked

\*Significant  $\chi^2$  deviation at the 0.1 level. \*\*Significant  $\chi^2$  deviation at the 0.01 level.

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# 3) Inheritance of a second leucine aminopeptidase locus and tests of its linkage with other lociy/

In dry soybean seed, only one leucine amino peptidase (LAP) anodal band was observed by acrylamide slab gel electrophoresis (Gorman et al., 1983). This band gradually declined in intensity and disappeared in all tissues about 10-12 days after germination. Three mobility variants (Rf's 0.59, 0.53, and 0.58) were observed in the band, which was controlled by a single locus (Gorman et al., 1983; Kiang and Gorman, 1983; Gorman, 1983).

A second LAP anodal band (Rf 0.80) was detected in all tissue of both G. max and G. soja plants about 8 days after germination. Cotyledons from 12- to 15-day-old seedlings were used to screen for the second LAP variant by acrylamide slab gel electrophoresis.

One activity variant and three mobility variants of the second LAP band were observed among 400 G. max cultivars and 140 G. soja accessions examined. The null activity variant found in cultivar 'Jefferson' did not show enzyme activity in any tissue. Based on the progeny of the crosses between 'Amsoy' and Jefferson, Jefferson x Amsoy and Jefferson and 'Wilson', genetic analysis indicated that the null was recessive to the active allele, and the F2 segregated into a 3:1 ratio (Table 1). Since there was no difference between reciprocal crosses, it was controlled by a nuclear gene. This second LAP locus is designated Lap2 to distinguish it from the first LAP locus (Lap1), and the null allele is designated as lap2.

Table 1. The  $F_2$  data segregating for leucine aminopeptidase activity at the Lap2 locus in soybean

Cross	Zymogram: Genotype:	Present <i>Lap2</i>	Null 1ap2 1ap2	χ² (3:1)	Р
Jefferson x Amsoy 1ap2 1ap2 x Lap2 Lap2		98	28	0.519	0.6
Amsoy x Jefferson Lap2 Lap2 x lap2 lap2		105	33	0.093	0.9
Total		203	61	0.505	0.6

The F2 dihybrids segregation data were used to test linkage relationships by the maximum likelihood method (Allard, 1956). The results showed that the Lap1 and Lap2 loci were unlinked (Table 1A), and the Am3 locus (Gorman and Kiang, 1978) was found to be inherited independently of the Lap2 locus (Table 2B). The F2 data from dihybrids involving the Lap2 and the hypocotyl color trait were examined. The F2 data showed that the purple hypocotyl color was dominant to the green, and segregated into a 3:1 ratio. The hypocotyl color is controlled by the gene pair  $(W_1 \ W_1)$  that also controls flower color by pleiotropic effect (Bernard and Weiss, 1983; Palmer and Payne, 1979). The linkage test indicated that the Lap2 locus was linked with the hypocotyl color locus  $W_1$  with 40.9±4.0 map units between them (Table 2C). Thus, Lap2 would belong to linkage group 8 (Palmer and Kaul, 1983). The estimate is based on a sample size of 268 plants, and a larger sample size is needed to make a more accurate estimate.

Table 2. The F<sub>2</sub> data from soybean dihybrids segregating for several gene pairs

				Pher	notype	freque	ncy	χ²
	Gene pair	Cross	Phase	а	b	С	d	(9:3:3:1)
Α.	Lap1-Lap2	Jefferson x Wilson	R	214	80	87	31	3.758
В.	Am3-Lap2	Amsoy x Jefferson	R	107	31	32	11	0.638
С.	Lap2-W <sub>1</sub>	Amsoy x Jefferson	R	154	50	39	25	6.653

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## 1) Mechanical separation of seed from male-sterile and fertile plants by seed $\frac{\texttt{size}}{}^*\!/\!/$

In soybeans, there are at least four recessive genes  $(ms_1, ms_2, ms_3, ms_4)$  that impart unconditional male sterility. A plant homozygous for any of these genes sets seed almost exclusively as a result of pollen transfer from malefertile plants. A consequence of male sterility is that seed from sterile plants tends to be larger than those from fertile plants (Burton et al., 1979; Carter et al., 1983). The reason for this effect is not well-understood. However, it is generally assumed to result from a reduced seed set per pod on male-sterile plants.

It occurred to us that this seed size differential might be used to simplify the annual maintenance of male-sterile and fertile isolines. A possible scheme for maintaining an isoline pair would include 1) a large "natural crossing block" which segregates for male sterility, and 2) insect vectors for adequate seed set on male-sterile plants. The block would be mechanically harvested in bulk at maturity. Seed from male sterile plants are separated out of the bulk based on large seed size. These large seed then become the planting seed for the next year's maintainer block.

Such a system requires less manual labor for harvest than our current system. In addition, this system requires no pollen identification of malesterile types. Pollen identification would become necessary in our present system if yields of male-sterile plants should be high. For example, 'Williams'  $ms_2$  isolines do not fit well in our current maintenance system because of high yields on sterile plants.

At this point, we should briefly review our current system for maintenance of male-sterile isolines. Typically, maintainer lines are half heterozygous and half homozygous recessive at the sterile loci and near isogenic at all other loci. To maintain the line it must be grown in an isolation block where insect pollen vectors will be available. At maturity, only the male-sterile plants are harvested in order to maintain the 1:1 fertile to sterile genotypic frequencies. A maintainer block is necessarily a random mixture of sterile and fertile plants because genotypically sterile seed cannot be distinguished from genotypically fertile seed at planting. Thus, sterile plants must be identified and removed individually from the block for threshing. For  $ms_1$  isoline pairs, male-sterile plants are usually identified visually by low seed set. For other male-sterility genes (especially  $ms_2$ ), pollen identification at flowering may be required.

It should be noted that Bradner (1975) patented an interesting method for hybrid seed production that involves seed size differential, although, in his system, a genotype with extruded floral stigmas rather than a male-sterile genotype serves as a female parent. (The extruded stigma presumably results in a high percentage of hybrid seed due to insect pollination and a low percentage of selfed seed.) To produce hybrid seed, a small-seeded female line is grown near a large-seeded male line. Because they result from pollination by the large-seeded male, hybrid seed are expected to be larger than selfed seed.

Bradner's system is dependent upon the existence of xenia for seed size. To date, there is little evidence of this effect in soybeans. Kilen (1980) found seed size to be mainly determined by the maternal parent. With no paternal influence, hybrid seed could not be distinguished from selfed seed in the system proposed by Bradner. Our system is different from that of Bradner's because his method depends on xenia for success. Ours does not.

It is clear that the feasibility of our system rests in large part on efficient mechanical separation of seed. With this idea in mind, we tested our ability to mechanically separate seed from male-sterile and fertile plants for two isoline stocks.

<u>Materials and methods</u>: Seven plants of Williams, a group 3 cultivar, and seven plants of its male-sterile  $(ms_2 \ ms_2)$  isoline were grown out-of-doors in pots at Raleigh, NC, in 1983. 'Forrest', a group 5 cultivar, was grown nearby as an additional pollen source. Seed from sterile and fertile plants were harvested separately at maturity to form 2 bulks.

N69-2774, a group 8 line, and its sterile  $(ms_1 \ ms_1)$  isoline were grown at Clayton, NC, in 1983. Male-sterile plants were bulk harvested from a maintainer block while fertile plants were bulk harvested from a separate block approximately 200 yards away.

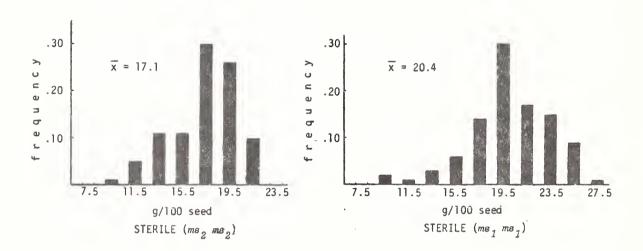
Two hundred seed were selected at random from each bulk. Each sample of 200 seed was passed through a series of hand-held screens. In addition, 100 random seed from each sample were individually weighed in order to obtain a distribution for seed weight.

Results: Mean seed size for male-sterile and fertile isolines differed by 3.9 grams per 100 seed in each isoline stock. However, the fertile and sterile isolines show considerable overlap in seed-weight distributions (Fig. 1). This overlap indicates that mechanized separation of seed from male-sterile and fertile isolines may be difficult in practice.

The isolines also were passed over a series of screens to test the feasibility of mechanical separation (Table 1). Results show that seed from sterile plants can be recovered in relatively pure form if a large amount of seed is discarded, and if seed from sterile and fertile plants are present in equal quantities. For instance, a 50-50 mixture of seed from male-sterile ( $ms_2$   $ms_2$ ) and fertile Williams plants could be screened to retain the largest 20% of the seed. This sample of largest seed would consist primarily of seed from male-sterile plants with only 2% contamination from fertile plants. A 50-50 mixture may, in fact, represent a "real life" situation for a bulk harvest of this isoline pair. (Yields of male-sterile plants are nearly equal to their fertile counterparts in some environments [Carter et al., 1983].)

By contrast, screen separation for male-sterile  $(ms_1\ ms_1)$  and fertile isolines of N69-2774 is not encouraging. Retaining the largest 20% of seed from a 50-50 mixture results in a seed sample from male-sterile plants which contains 10% contamination from fertile plants. In practice, however, contamination would be much higher; a typical bulk from a natural crossing block is at best a 20-80 mixture of seed from male-sterile and fertile plants. (Seed set is always low on male-sterile plants conditioned by the North Carolina  $ms_1$  gene.) In this more typical situation, contamination would reach over 30%.

WILLIAMS N69-2774



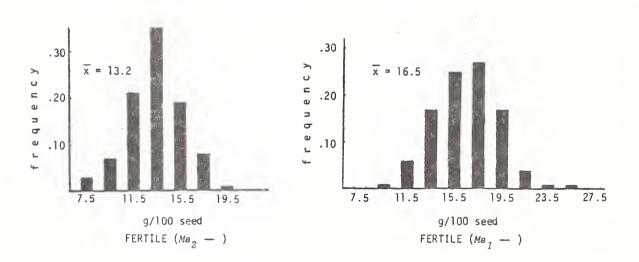


Figure 1. Seed weight distributions for Williams  $\underline{ms}_2$  isolines, and N69-2774  $\underline{ms}_1$  isolines.

Table 1. Mechanical separation of seed samples from two sets of fertile and male-sterile isolines

	Willi	ams	N69-2	774
Screen size	Sterile (ms <sub>2</sub> ms <sub>2</sub> )	Fertile	Sterile (ms <sub>1</sub> ms <sub>1</sub> )	Fertile
19 <sup>a</sup>	0 <sub>p</sub>	0	0	0
18	5	0	35	4
17	40	1	38	29
16	36	13	18	35
15	13	47	6	25
14	5	31	1	5
13	1	7	2	2
12	0	1	0	0
	100%	100%	100%	100%

<sup>&</sup>lt;sup>a</sup>Diameter of round holes expressed in 64th of an inch.

In summary, the inferences from our results are limited at best. Both environment and genetic background seem to have a great effect on the ability to mechanically separate seed from male-sterile and fertile isolines. It may be safe to assume, however, that mechanical separation will not be easy in many cases. The ultimate utility of this method awaits further testing.

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<sup>&</sup>lt;sup>b</sup>Percent of sample which rested on top of indicated screen but passed through the next larger screen.

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### 1) Resistance to anthracnose

Anthracnose, caused by *Colletotrichum dematium* (Pers. ex Tr.) Grove var. truncatum (Sahw.) Arx., can be found in most U.S. soybean producing areas. It is the most prominent disease in much of the southern U.S. and for the Texas Gulf Coast. In recent years, estimates of yield losses caused by anthracnose in the southern U.S. have exceeded 28 million bushels per year, a 2.5% loss in potential yield (Sturgeon, 1980). Anthracnose has accounted for about 13% of all yield losses attributable to fungi, bacteria, viruses, and nematodes (Sturgeon, 1980).

Anthracnose control is achieved by foliar application of various fungicides. Neither resistance nor tolerance has been reported. The development of cultivars with resistance to anthracnose could increase yields and profitability. Partial resistance or tolerance could reduce the need for/or number of fungicide applications, thus helping reduce the costs of production.

From 1981 to 1983, approximately 1500 accessions of maturity group V through VIII from the USDA Germplasm Collection were screened for resistance to anthracnose. In field trials at Beaumont, two-row plots of each germplasm entry were observed and rated for pod and stem symptoms from natural infection. Plants were rated on a scale of 1 to 9, taking into account extent of lesion development on both pods and stems of mature plants. The rating was made on at least one occasion after R8 by two observers, with the average value being recorded. Anthracnose is severe at this location and good uniform infections occur without the use of spreader rows or inoculations. Entries with low levels of symptom development were evaluated in replicated trials the following year.

Table 1 lists the plant introductions rated as resistant from the 1983 second cycle of screening. These lines were rated as resistant for at least two and in some instances for as many as four years. The selected plant introductions do not include any entries from maturity group V or VI, which does not necessarily indicate a lack of resistance to typical pod and stem lesion formation in material of this maturity. The 1983 season was extremely wet, resulting in a serious and atypical outbreak of anthracnose on early maturing material. Symptoms included leaf abscission accompanied by petiole retention, green stems at maturity, and pod blanking. No differences were observed between any material of maturity groups V or VI in their reaction to these severe symptoms.

This screening program will continue with approximately 500 new plant introductions added each year. Lines rated as resistant will be reevaluated continually.

Table 1. Reaction of soybean germplasm to anthracnose infection

Cultivar or PI No. <sup>a</sup>	Maturity group	Disease rating b	Cultivar or PI No.	Maturity group	Disease rating
Dare	V	6.5	229.358	VII	3.0
Davis	VI	6.5	283.326	VIII	4.0
Bragg	VII	5.5	285.091	VIII	4.0
Dowling	VIII	6.0	285.095	VIII	4.0
171.451	VII	3.5	309.658	VIII	4.5
183.929	VII	4.0	319.533	VIII	4.5
183.930	VII	4.5	341.252	VIII	3.5
189.402	VII	3.5	374.172	VIII	4.5
200.452	VII	4.0	374.177	VIII	4.5
200.455	VII	4.5	376.845	VIII	3.5
200.456	VII	4.5	379.623	VIII	3.5
200.462	VII	4.0	416.764	VIII	3.0
200.465	VII	4.0	416.886	VIII	3.5
200.466	VII	3.5	417.061	VIII	3.0
200.476	VII	4.5	417.117	VIII	4.0
200.484	VII	3.5	417.134	VIII	3.5
200.532	VII	4.5	417.208	VIII	4.0
200.539	VII	4.5	417.215	VIII	3.5
210.351	VII	3.5	417.470	VIII	3.0
219.652	VII	4.5	417.566	VIII	4.5
224.273	VII	3.0	417.569	VIII	4.0
227.224	VII	4.0			

<sup>&</sup>lt;sup>a</sup>Dare, Davis, Bragg, and Dowling were included as the check varieties.

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 $<sup>^{</sup>m b}$ Disease rating is on a scale of 1 to 9 with 9 being most severe.

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### 1) Soybean floral ecology and insect pollination/

The cultivated soybean (Glycine max (L.) Merrill) is an herbaceous annual, unknown in the wild, with uncertain ancestry. Most believe that its origin was in Eastern Asia, probably Northeastern China, where it was first cultivated about the 11th century B.C. (Probst and Judd, 1973). Like corn, the soybean may have been selected and bred by ancient man from a more primitive form that was different in growth habit and floral development. Existing primitive soybeans and the cultivated soybean may be the same species (Probst and Judd, 1973) or perhaps, the ancestral soybean species has been lost (R. Bernard, personal communication).

Among the traits that may have been altered through man's selection and breeding is the soybean's natural pollination syndrome (Rubis, 1970). This could have occurred because selection of the cultivated soybean out of its wild parent was likely carried out in agricultural areas relatively free of insect pollinators. Hence, unwitting selection against bee-pollinated types in the modern soybean would have been made. Couple this conjecture with numerous observations of good crop yields in the apparent absence of bees, and it is not surprising that many believe that bees cannot influence soybean yields (see Erickson, 1975a). However, those who hold this view overlook four key points: 1) that the structure of soybean flowers definitely encourages bee visitation with concomitant pollination; 2) that bees forage extensively in soybeans; 3) that, in the past, studies regarding the relative effect of pollinating insects on soybeans were usually conducted without knowledge of pollinator populations at the study site(s); and 4) that, in the absence of an identified wild progenitor, there has been no consideration given to the pollination of the ancestral parents of the cultivated soybean. Note: We now know that a related species, Glycine falcata Benth., is insect pollinated (Anderson et al., 1983).

The subject of honey bee (Apis mellifera L.) foraging on soybeans has long been immeshed in controversy, and debated publicly for over 50 years (see Erickson, 1975abc). There are those who have steadfastly maintained that bees do visit soybeans to gather nectar and pollen (and, perhaps, pollinate them), while others hold the opposite view with equal conviction. Both observations may, in fact, be accurate, as will become evident.

A somewhat complex picture of interactions between soybean cultivar and environment seems apparent. At certain locales and under certain circumstances, foraging by bees, the principal insect pollinators, on a soybean cultivar may be extensive; at other locales, it may be limited or nonexistent. As a result, floral nectar may or may not be secreted and bean yield may or may not be affected.

Soybean flowers and honey bees — As I and others (Abrams et al., 1978; Erickson, 1975c, 1976; Erickson and Robins, 1979; Jaycox, 1970; Kettle and Taylor, 1979; Mason, 1979; Sheppard, 1975) have pointed out, certain cultivars of soybeans are more extensively visited by bees and produce greater quantities of nectar and aroma than others. Moreover, since soybean cultivars are restricted geographically to narrow latitudes based upon rate of maturation, those cultivars known to be preferred by bees in one area may or may not produce nectar or aroma and, therefore, may not be attractive to bees at other localities. Hence, when referring to soybeans, one must consider the specific cultivar involved. Cultivars grown within the range of their maturity group seem to elicit the most intense bee/flower interactions.

Other factors further contribute to optimal floral development and pollinator foraging. Soybeans have long been, and in some areas are still, considered a secondary crop, grown only in deference to other row crops, such as corn and cotton. For this reason, perhaps more than any other, the best soybean husbandry practices, such as optimizing plant density and nutrient fertilization, have not always been followed. For example, many farmers do not follow existing recommendations and adjust their planter to narrower rows for soybeans after planting corn or cotton. And, frequently, farmers plant their best land to other crops, giving their fields of lesser productive capacity over to the beans. Poor crop husbandry contributes to reduced bee visitation due to altered foraging cues and rewards (Robacker et al., 1982b; Robacker et al., 1983).

The flower The soybean flower is variable in size; some are long and relatively narrow, while others are short and broad. Petal color ranges from white through mauve to purple, yet most cultivars possess pigmented flowers. Each zygomorphic flower has five petals. The standard petal is bound on either side by a smaller wing petal, while two tightly clasped ventral keel petals partially enclose the sexual column (Carlson, 1973; Erickson and Garment, 1979).

Previously published depictions and descriptions of soybean nectaries create confusion because of their inaccuracies. It is quite clear that soybean blossoms possess most, if not all, anatomical characteristics of beepollinated (anthophilous) flowers, including: 1) nectar guides (both in the visible and ultraviolet spectra); 2) a characteristic aroma (detectable at higher temperatures, e.g., above 27°C); 3) a tongue channel and guide (for pollinators -- probably bees); and 4) a highly differentiated discoidal nectary (Erickson and Garment, 1979) that produces substantial quantities of nectar. Preliminary data suggest that floral aromas may inform pollinators of flower pre-readiness, readiness, and post-readiness for visitation (pollination) with separate chemical messages (Robacker et al., 1982a). Further studies are now underway to identify and bioassay flower volatiles and to confirm this concept. The structure of the flower and the approach behavior of the foraging bee ensure that bees will contact the sexual parts of the flower whether gathering nectar or pollen (Erickson and Garment, 1979). Yet, in cool climates or during cool weather, the flowers of most soybean cultivars are cleistogamous and, hence, are inaccessible to bees.

Two to 35 flowers are borne in racemes at the nodes of the stem and branches (Carlson, 1973). They first open at the base of the raceme and then open progressively upwards. Each soybean flower is open for only a single day (E. Erickson et al., unpublished data; Severson, 1983), but from one to 13 may be open simultaneously on a raceme depending upon the cultivar (Erickson,

1975a). When the leaf canopy is moved aside and several flowers are open simultaneously on each raceme, floriferous cultivars appear quite showy. The number of flowers produced per hectare is highly variable among cultivars. Sheppard et al. (1979) estimated a range of 1.3 to 4.1 million flowers per ha per day in Illinois. Generally, soybeans do not compete well with other legumes for the attention of bees, due to the relative numbers of flowers per hectare; other legumes, such as alfalfa and clover, may have up to 10 times the number of flowers per ha and greater numbers of flowers per cluster. A soybean field is usually in bloom for 4 to 6 weeks and in agricultural areas where early and late adapted cultivars bloom in succession, a 6-9 week flowering period ensues.

Nectar Soybean blossoms have functional nectaries (Erickson and Garment, 1979). Each flower of most cultivars produces only slightly less nectar than alfalfa in northern regions. Sugar concentrations in soybean nectars are 5-10% higher than those of alfalfa when growing conditions are favorable (Erickson, 1975c; Severson, 1983). We see similar variability among cultivars in nectar production and attractiveness to bees in both southern and northern regions of the U.S.

In the central United States, soybean nectar production and bee visitation occur between 0900 and 1500 h each day. Peaks in these activities, like the time of day when the flower is first fully open, may vary, depending upon the cultivar and local weather conditions. Soybean nectar volume per flower, greatest in warmer climates, varies significantly among cultivars, ranging from none to 0.2 microliters per flower, with some flowers having as much as 0.5 microliters (Erickson, 1975c; Severson, unpublished data).

Several workers have examined soybean nectar and reported a mean nectar sugar content of 37.0 to 45.0% (Erickson, 1975bc; Jaycox, 1970). Kettle and Taylor (1979) reported a 39.5% sugar concentration for the cultivar 'Forest' in Kansas. Severson (1983) found that the total carbohydrate content in soybean nectar varied from 301 to 1354  $\mu g/\mu l$  of nectar and from 15 to 134  $\mu g$  per flower. Floral sugar concentration increased but volume decreased with time of day and temperature. Sugar ratios (i.e., fructose/glucose/sucrose) differ among soybean cultivars, as well as with time of day within a cultivar (Severson, 1983). Severson (1983) noted no differences in carbohydrate content between purple and white flowered cultivars. Nectar production from flower to flower appeared to be most consistent in volume and carbohydrate content (Erickson, 1975c) among white flowered cultivars; hence, white flowered cultivars were judged more attractive than purple flowered cultivars. But, later work by Mason (1979) and Severson (1983) seems to dispel this notion.

Sheppard (1975) and Sheppard et al. (1979) reported a mean sugar concentration of 39.9% (range 13.0 to 60.0%) for nectars of four soybean cultivars as taken from the honey stomachs of bees. Here, differences in sugar content among sampling dates varied as much as that among cultivars and fields. O'Keefe Van Der Linden (1981) reported sugar concentration in the stomachs of honey bees foraging soybeans in Iowa to be 28.0% (range 20.0-33.0%) in 1979 and 51.0% (range 38.0-63.0%) in 1980.

Pollen Honey bee collection of soybean pollen is highly variable as is a cultivar's ability to produce quantities of pollen. Some cultivars produce twice as much pollen as others (Palmer et al., 1978). Erickson (1975abc) and others (see Erickson, 1975a) have noted that little soybean pollen may be

gathered by bees in some areas. However, I (personal observation) and Jaycox (1970) determined that soybean pollen comprised over 50% of the total quantity of pollens gathered by many bee colonies in Arkansas and Missouri and Illinois. Soybean pollen pellets taken from the corbiculae of foraging bees are easily recognized by their grey-brown color, small size, and compaction.

The bees Many species of bees, including honey bees, forage soybeans for nectar and pollen. Honey bee populations may exceed a density of 1 bee per meter of row during peak foraging (Erickson, 1975c). Sheppard et al. (1979) found that Caucasian bees gathered a greater percentage of soybean pollen (0-54% by day) than did Carniolan and Italian bees. Rust et al. (1980) reported 29 additional species of bees that forage soybeans in three regions of the United States. They reported further that others had identified several species of bees foraging on soybeans: Missouri, seven species; North Carolina, six species; Indiana, three species. The contributions of bees other than honey bees to soybean yields are unknown.

Soybean honey production Beekeepers, particularly those in the central and southern United States, have been obtaining substantial yields (70-90 kilograms per colony) of light amber honey from soybeans for decades (Erickson, 1975a; Warren, 1983). In so doing, they have identified those agricultural lands where ample soybean honey production can be expected, as well as those areas that are of unreliable or nonexistent productivity (Erickson and Robins, 1979). There is little doubt that many beekeepers unknowingly harvest large quantities of soybean honey. For example, O'KeefeVan Der Linden (1981) found soybean pollen in 15 of 15 honey samples taken from eight Iowa counties in 1979, and in 46 of 48 samples from 35 Iowa counties in 1980. Only samples from the northeast corner of the state failed to show soybean pollen in the stomach contents of bees. Often, soybeans are not exploited by beekeepers for the production of this honey, which has a distinctive aroma and flavor and is easily identified with experience (Erickson, 1975abc).

Nectar production in soybeans, as in other plants, is dependent in part upon weather (Erickson, 1975c; Severson, 1983). During cool periods, mature flowers remain partially or fully closed and have no nectar. In 1973, I was able to observe that plants in more northern climates (e.g., Wisconsin) required three days to recover the ability to produce nectar following a period of cool weather, even though the flowers were open each day. The quantity of nectar produced per flower following cool weather usually will not reach the level that was present during the preceding favorable period. Temperatures above 22-24°C are required to insure nectar production (Erickson, 1975bc).

Intuitively, the most vigorous plants given optimal plant husbandry should produce the greatest quantities of nectar. Since most of the basic components of nectar, including sugars, are products of photosynthesis, the healthiest plant receiving the maximum amount of light and grown in the most suitable soil is likely to be the greatest producer of flowers with quality nectar and aroma and, thus, the most attractive to foraging bees (Erickson and Robins, 1979; Robacker et al., 1982ab; Robacker et al., 1983; Shuel, 1975). Soybean seed yields are sensitive to the presence and availability of certain soil nutrients, soil pH (a pH level of 6.0-6.5 is considered optimum), and soil moisture, as well as sunlight. For example, soybean yields are significantly reduced when a crop is grown on acid soils that have a pH level below 6.0. Soil nutrient availability (particularly phosphorus and potassium) is reduced with increasing soil acidity. Various soil micronutrients are similarly affected. At low pH, potassium, phosphorus, calcium, and boron interact with

one another, inducing deficiencies. Thus, optimal soil pH and soil fertility are vital to the physiological well-being of the plant (Erickson and Robins, 1979), its ability to produce flowers, nectar and aroma (Robacker et al., 1982a; Robacker et al., 1983) and, probably, its yield response to bee pollination.

Soil texture, too, is important to soybeans, since it affects nutrient retention, soil moisture availability, and root penetration. In southern Missouri, sandy, coarse loamy, and coarse silty soils provide the least amount of available water to the plant followed by the clayey soils; the fine loamy and fine silty soils supply the greatest amount. Moisture stress reduces photosynthesis as well as flowering and pod filling. Coarse soils are readily leached, and so are usually acid and low in fertility (Erickson and Robins, 1979). Fertility can be restored to these lands, but, unless good crop husbandry is practiced, nectar secretion and resultant honey production is likely to be poor (10-20 kg per colony). Heavier soils are less acid, more fertile, and retain their productivity partly because they are difficult to till. As a result, crop yields are usually high (2.6-4.3 kl/ha), and our experience has shown that high soybean honey yields (90 kg per colony) also can be expected. Nectar secretion in various other plant species has been shown to be adversely affected by low soil moisture availability, low soil nutrient availability, and low pH. Nectar secretion is generally low on soils with either too much or too little drainage (Erickson and Robins, 1979; Severson, 1983).

Studies conducted in a controlled-environment facility demonstrated that plant/flower characteristics, indicating greater plant vigor, were optimal at the intermediate day and night air temperatures (28 and 22-26°C), the higher soil temperature (28-32°C), and the higher (175 ppm) and lower (15 ppm) soil concentration of nitrogen (N) and phosphorus (P), respectively. Bioassays showed that honey bees preferentially visited soybeans that had more flowers which produced greater quantities of nectar (Robacker and Erickson, 1984). The predominant environmental factors contributing to attractiveness of soybeans to bees were moderate and high air temperature and high and low soil concentration of N and P, respectively (Robacker et al., 1983).

Bee pollination of soybeans Soybeans are classified as self-fertile and automatically self-pollinating. It is said that pollination may occur before the blossom opens (Carlson, 1973). Moreover, large numbers of fertilized and unfertilized flowers (more than 75% in some cultivars) drop off the plant and do not set seed (Carlson, 1973). Thus, it would appear that soybeans normally set a full complement of seed and, therefore, have little biological need for insect pollination among cultivars and, hence, little need for the kind of floral development characteristic of insect-pollinated plants. Indeed, many argue that such is the case. Others of us disagree. Even though the soybean retains a high level of heterosis because it is a disomic polyploid, some outcrossing would be beneficial. The question is: How much interfloral pollen transfer both within and between cultivars occurs naturally?

One must now wonder whether the earlier observation that soybeans self-pollinate before the flower opens may have involved a misunderstanding of cleistogamy and the fact that soybean blossoms are open for only a single day. Recently, Robacker et al. (1982a), working in a controlled environment, found that only 33% of the 'Mitchell' soybean flowers examined were completely self-pollinated 3.5 hours after the onset of photophase; 58% were self-pollinated 6.5 hrs after the photophase began. These results suggest that early in the day soybeans exercise a cross-pollination strategy that is followed by a self-pollination strategy later in the day. Follow-up field studies are now needed

to examine this aspect of floral development under field conditions. If corroborated, we should expect that the temporalization of these strategies may vary with the cultivar's relative abundance of pollen (Palmer et al., 1978) and with other factors as well.

Cross pollination
at from less than 0.5% to 35% (see Caviness, 1970; Erickson, 1975ab; Koelling et al., 1981; Sadanaga and Grindeland, 1981) with outcrossing most evident among lines in which pollination occurs the morning the flower opens (see also Robacker et al., 1982a). Note: See later section on hybrid soybeans for additional evidence of natural cross-pollination.

Outcrossing rates are a real measure of specific field circumstance, but one can neither consider them to be a measure of overall bee visitation nor a measure of pollination within a soybean genotype. While distance between pollen and seed parent and other factors have been shown to be related to outcrossing rates (Carter et al., 1983a; Nelson and Bernard, 1979), the primary limiting factors are undoubtedly the number of bees present and the level of fidelity to a single genotype practiced by individual bees. These must be determined, then bee pollination both within and between genotypes must be measured to ascertain the net result of pollinator activity.

Unfortunately, the true meaning of rates of outcrossing in insect-pollinated plant species has been misinterpreted by many people. Foraging honey bees normally discriminate among and retain a fidelity to a single floral source. And, so, just as honey bees discriminate among plant species, so do they often discriminate among cultivars or genotypes that differ in foraging cues (e.g., flower color and aroma) or reward within a species (see Erickson, 1983). This is true for soybeans (Severson, 1983). Outcrossing in soybeans is most frequently monitored between white and purple flowered cultivars (color differences that bees readily discriminate between) due to ease of evaluation based upon seedling hypocotyl color (e.g., Burton and Carter, 1983; Carter et al., 1983b). The fact that intraspecific crossing is usually low, when measured in this fashion, is hardly surprising given the well-known floral constancy of foraging bees. Apiculturalists would be surprised if it were otherwise!

Soybean yield increases Recently, studies have shown that bees may increase soybean yields by as much as 20% (or more) for plots caged with bees vs. caged without bees. Erickson (1975a) demonstrated a yield increase of 13.9% for the cultivar 'Corsoy' in 1971 and 5.2% and 16.3% for 'Hark' in 1972 and 1973 in Wisconsin. In the Mississippi Delta, Erickson et al. (1978) obtained a combined yield differential of 21.6% on the cultivar 'Pickett' at two study sites in Arkansas and Missouri in 1975. Here, significant differences in the numbers of filled and empty pods were also noted. These differences were attributed to increased pod set, since seeds per pod and weight per seed did not vary. C. E. Mason (unpublished data), in Delaware, obtained three-year yield increases in cages with bees of 7.8%, 2.2%, and 15.8% for the cultivar 'Williams' and 16.0%, 2.7%, and 14.3% for the cultivar 'Essex' in 1978-80. respectively (data for the years 1978 and 1980 are significant at the 5% level). Kettle and Taylor (1979) obtained yield increases of 5.1%, 19.9%, and 36.0% ( $\bar{X}$  = 20 percent -- significant at the 5% level for pooled result), respectively, at each of three study sites in Kansas for the cultivar 'Forrest'. A second cultivar, 'Woodworth', grown at yet another site, had a yield differential of 10% (significant at the 2% level). In Brazil, Juliano

(1976) noted a significant increase in both the number of pods and weight of total seed (37.9% and 40.1%, respectively) in open plots versus plots caged to exclude pollinators. In Italy, Pinzauti and Frediani (1981) obtained yield differentials in excess of 100%, in both seeds and pods, with bee pollination on two cultivars ('Grangeneuve' and 'Hei-iee-Jin'). Unfortunately, they, like Juliano, caged only the plot without bees and, thus, failed to evaluate the effect of the cage which, on soybeans, can be great (Erickson et al., 1978). Hence, while their conclusions may be correct, their exceptional results are likely due in part to the effect of the cage.

In open field trials in Arkansas and Missouri, Erickson et al. (1978) obtained significant yield differences between that side of the field near the apiary versus the far side of the field. These data compare favorably with those of Abrams et al. (1978): both data sets show a high yield near the bees (5-15 m from the apiary), a still higher yield at 20-35 m and then a progressive decline at greater distances from the colonies (Table 1). Similar patterns are common in other insect-pollinated crops.

Table 1.

Distance from	Average number of	seeds per sample <sup>a</sup>	kg/ha <sup>b</sup>
apiary	Arkansas	Missouri	Indiana
5-15 meters	785 a <sup>c</sup>	836 a	2654 a
20-35	839 a	931 a	2719 a
50-65	619 Ъ	776 a	2473 a
85-100	630 Ъ	529 Ъ	2350 a
115-150	594 Ъ		2350 a

<sup>&</sup>lt;sup>a</sup>Each sample consisted of all seeds from 10 plants.

In other studies, soybean yield differences due to bees have not been observed. E. H. Erickson and E. S. Oplinger (unpublished data) were unable to show significant yield differences in five cultivars ('Hark', Williams, 'Illini', 'Wayne' and 'Mukden') over three years, although caged treatments with bees were usually slightly above those caged without, in total beans and pods. Some cultivars during some years did show a significant difference in numbers of beans per pod. These studies were conducted in an area in southern Wisconsin on land of higher productivity than the earlier trials (Erickson, 1975a) with Corsoy and Hark. Sheppard et al. (1979) were unable to demonstrate significant yield differences in open field studies in Illinois of 'Amsoy' and Williams, although their data show a slight trend of yield decline (similar to those in Table 1) with increasing distance from the apiary for Williams.

bCombined field data (Abrams, 1977).

<sup>&</sup>lt;sup>C</sup>Within columns, values followed by different letters are significantly different at the .05 level.

Soybean cultivars are often identified as being determinant (cease vegetative growth before beginning to flower) or indeterminant (flower while continuing to grow). In reality, all soybeans are indeterminant, but individual cultivars vary in their tendency towards determinancy with later maturity group cultivars tending to be more determinant (R. Bernard, personal communication). Even so, unlike Sheppard(1975), Sheppard et al. (1979) and Mason (1979), I have yet to discern differences in foraging by bees or yield response resulting from bee pollination that can be explained based upon level of determinancy at flowering.

Hybrid soybeans The development of hybrid soybeans is a topic of interest both for beekeepers and plant breeders, as well as others in agriculture (Erickson, 1979). Substantial interest was generated after Brim and Young (1971) reported the discovery of genetic male sterility in soybeans. Bradner (1977) attempted to produce hybrids using a genetic characteristic for open flowers that would enhance outcrossing. It was suggested that hybrid soybeans would be available in five to ten years. The present status of hybrid soybeans is uncertain, as genetic male sterility presents some difficult problems if it is to be considered for development of commercial hybrids and the work initiated by Bradner is not progressing as expected. Cytoplasmic male sterility in soybeans is as yet unknown.

Some studies have shown that there may be a relatively high level of cross pollination (62%) in at least one genetic male-sterile soybean (Sadanaga and Grindeland, 1981). Koelling et al. (1981) found a significant increase in seed set on male-sterile soybeans caged with bees versus caged without bees (honey bees, 39 seeds per plant; alfalfa leafcutter bees, 40 seeds per plant; no bees, 3 seeds per plant). Similarly, Nelson and Bernard (1979) obtained fewer than 10 seeds per plant at 15 m from the pollen parent to more than 70 seeds per plant at 1.5 m in studies to determine the relationship of distance from pollen source to pollination of male-sterile soybeans. In these studies, the male-sterile plants produced an average of 27 seeds per plant -far less than the normal production of 70 to 100 + seeds per plant (Erickson, 1975a; Erickson et al., 1978). Again, these data must be interpreted carefully considering the discriminative foraging behavior of bees discussed earlier. Inadequate pollination is a major factor limiting the production of hybrid soybeans (Nelson and Bernard, 1984), just as it has limited hybrid production in other crops (Erickson, 1983).

Whether hybrid soybeans will become a commercial reality remains to be seen. Some researchers feel that it is just a matter of time; others think it unlikely that hybridization will ever become sufficiently practical for commercial seed production. Certainly, hybridization would contribute substantially to the research programs of plant breeders by reducing the necessity for hand-crossing and for obtaining large-scale outcrossing for recurrent selection (Burton and Carter, 1983; Carter et al., 1983a). Meanwhile, forthcoming soybean flower-pollinator data will enhance our knowledge of the pollination ecology of entomophilous plants. If hybrid soybeans become a reality, plant breeders must pay strict attention to floral characteristics and include selection for pollinator cues and rewards to ensure floral compatability between seed parents in their breeding programs (see Erickson, 1983; Rubis, 1970). Also, they should carefully evaluate grossly abnormal flower mutants (see Erickson et al., 1982ab; Johns and Palmer, 1982) in the light of pollinator requirements.

Conclusions The data presented indicate that bees produce substantial honey crops from soybeans and may increase soybean yields in some fields/localities, but not in others. Hence, the convictions of those on both sides of the issue appear equally valid. Only intransigence and imprecise descriptions of circumstances predisposing each conclusion can be faulted. Regardless of opinions to the contrary, many soybean growers continue to encourage beekeepers to locate apiaries near their fields and report increased yields with bees present.

Differences in soybean honey production and soybean yield due to bee pollination seem attributable in part to heritability factors and geoecology. Interpretation of all bee/soybean data suggest that greatest honey yields occur on the most productive soils in warm climates, while soybean yield increases resulting from insect pollination have been highest on poorer soils. Further research is needed to clarify these hypotheses. Some cultivars are more attractive to pollinators than others. Many cultivars have yet to be studied in this regard. Bees rarely visit soybeans in geographical areas with low median temperatures because soybean flowers do not open or produce nectar and aroma in these areas. Studies are needed to ascertain the nature of bee/flower interactions for each agricultural zone. Perhaps, as new knowledge is developed, new avenues can be pursued to maximize soybean yields for growers and honey yields for beekeepers.

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## 1) Some characteristics of lectins in soybean seeds

Natural complex of proteins, fats, carbohydrates, mineral salts and vitamins in legume seeds makes them nonsubstituted in establishment of stable feeding base for livestock. In human diet use of legumes is increasing, too. But some seed substances reduce nutritive and feeding qualities of legumes very much. The main role in this reduction belongs to inhibitors of proteolytic enzymes, cyanogenic glucosides and lectins (Benken and Makasheva, 1977; Golinskaya et al., 1981; Konarev, 1975; Sjödin et al., 1981).

Recent experiments demonstrate that uncooked legume meal is unable to maintain normal growth of animals because of some proteins, named phytohemagglutinins or lectins. They are very active biologically and able to unite with erythrocytes of blood and other cells, inducing their agglutination. The fact is that 2-10% of legume seed proteins are lectins, and it is necessary to keep in mind their biological function in nutritive and feeding meaning. Lectins are frequently toxic to animals. Therefore, they are of interest in relation to the poor nutritive value of certain plant proteins.

It is known that lectins from seeds of some legume cultivars are toxic in intraperitoneal injection, significantly reduce the growth, and become the cause of animals diseases, when uncooked seeds or untreated meal are incorporated into the daily diet. The hemagglutinating activity of legume meal extracts can be considered as an indicator of their nutritive and feeding value (Liener, 1974; Rackis et al., 1979).

The toxicity of legume seed lectins was not destroyed completely during boiling or autoclave treating; lectins were resistant to dry air-heating, too (Antunes and Sgarbieri, 1980).

There are various proposals on the role of lectins in plant life. It is known that they have protective function to prevent destruction of vegetative mass and seeds by pathogenic fungus, bacteria, and viruses, as by insects and pests (Albersheim and Anderson-Pronty, 1975; Bhuvaneswari, 1981; Jansen et al., 1976; Pueppke et al., 1978). It is known that lectins are very important for inoculation legume plants by rhizobium bacteria (Bohlool and Schmidt, 1974; Dazzo and Hubbel, 1975; Lee et al., 1980). Lectins are characterized by high and diverse biological activity, which depends on their influence on membranes and genetical apparatus of cells (Liener, 1976).

It is understandable now that study of lectins in feeding and nutritive legume cultivars is very prospective path in the breeding of these plants. In some countries, the experiments of this kind are made intensively (Pull et al., 1978; Sjödin et al., 1981; Stahlhut et al., 1981).

In our experiments, we have studied 20 cultivars of soybean. Lectins are isolated by divided ethanol fractionation (Rigas and Osgood, 1955). Meal was treated by ether sulfurious for 24 hr to remove fat. Proteins, after deetherification, were extracted in acided (pH 4.5) distilled water (1:8) for 2

 $<sup>^{\</sup>circ}$ 1984 by the authors

hr at  $37^{\circ}$  and for 2 hr at  $4^{\circ}$ C. Extract was centrifuged. In order to get perfectly clear solution, and also to concentrate the lectin somewhat, the active protein was precipitated at pH 4.5 by the addition of ethanol to 40--76% at  $0^{\circ}$ C, after having first thrown out and discarded a relatively inactive precipitate with ethanol at 30%. The precipitates were dried lyophilically. It must be pointed out that it was amount of a nonspecific, nonagglutinating protein, that was taken up (Boyd, 1970).

Total content of protein in meal was determined by the method of Lowry et al. (Bailey, 1965) using bovine serum albumin as standard and the quantity of agglutinating proteins was calculated as percentage to total protein content.

Agglutinating activity was studied by using standard methods of immunology. A 1% saline suspension (0.15 M NaCl) was prepared from freshly clossed human blood after it had been washed 3 times with 0.15 M NaCl. 0.05 ml of the lectin solution (1 mg/ml) was mixed with 0.05 ml of a 1% suspension of erythrocytes on a microscope slide, the mixture was incubated 30 min and the degree of agglutination read microscopically.

The assay of hemagglutinating activity was performed by mixing two-fold serial dilution of the lectin with an equal volume of 1% erythrocyte suspension. The activity was expressed as HA titer, the reciprocal value of the maximal dilution at which hemagglutination was observed.

The active precipitate was dissolved in 0.01 M phosphate buffer (pH 7.1) and analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Weber and Osborn, 1969).

Protein staining: After electrophoresis, the gels were removed from the tubes, immersed in 0.04% Coomassie Brilliant Blue G 250 in 3.5% perchloric acid for one day (Reisner et al., 1975), destained in acetic acid/ethanol/water (10:25:65) and then stored in 7% acetic acid.

This method also was used for molecular weight determination of studied proteins, using their electrophoretic mobility compared with protein markers (bovine serum albumin, polyedren protein, RNA-ase).

The lectins of various soybean cultivars were divided visually into two types: "meal-like" as meal mass, and "glass-like" as sugar sand. The maximum agglutinating activity was determined in protein fractions which precipitated in 50 and 76% of ethanol (Table 1). Isolated lectins agglutinate human erythrocytes of all blood groups in system ABO (group A O B), erythrocytes of rabbit, rat, pigeon, hen, etc.

Protein content in soybean seeds was  $30\pm2\%$  and only in four cultivars it was higher than 32% (Table 2). The quantity of lectins was between 1.064-4.06% in a meal or 3.38-12.78% to total protein content. The maximum content of lectins was found in seeds of European cultivars, the minimum - in American and Far-Eastern cultivars, and in Brazilian samples. Similar results were found by us recently during examination of 26 cultivars (Golinskaya et al., 1982). In this study, high content of lectins was in soybean seeds of European and Ukrainian breeding, and low was in American cultivars and in some samples from China. Some European cultivars had lower lectin content. No correlation between total protein and content of lectins was found (r = 0.12).

It is known from literature that there are numerous forms of lectins (Goldstein and Hayes, 1978). Electrophoretic analysis of agglutinating protein fractions, which precipitate at 50-76% of ethanol, pointed out the electrophoretic heterogeneity of total lectins, and certain differences in quantity and

Table 1. Hemagglutinating activity of protein fraction, isolated by ethanol fractionation from soybean seeds

			Etl	nanol, perce	ent	
Variety	Origin	30	40	50	60	70
Provar	USA	+- <sup>a</sup>	_	+-	_	++
Altona	Canada	+-	_	++	+-	++
Manchurian	China	_	_	+-	-	+-
Sal'ut 216	Far East	++		+++	+-	+++
Violetta	Roumania	+-	+-	+++	_	+++
Hungarian 48	Hungary	++	-	++++	-	1-1-1

<sup>&</sup>lt;sup>a</sup>The numbers of signs (+) signify strength of agglutination, +++ being the strongest (all the erythrocytes stuck together in 1 large clump). Negative reactions are recorded as -.

Table 2. Content of total protein and lectins in seeds of different soybean varieties

Variety	Origin	Total protein %	Lectins in a lot %		Titer of agglutina-tion
Rouest	Hungary	29.7	2.28	7.67	1:32
Heimkraft	GDR	30.4	1.38	4.54	1:8
Pavlikeni	Bulgaria	28.9	3.10	10.73	1:64
Pshebedonska	Poland	31.9	4.06	12.72	1:16
Zora	Czechoslovakia	34.8	2.86	8.22	1:16
Carona	Hungary	31.0	2.94	9.48	1:128
Soy seto	Hungary	31.6	1.36	4.30	1:64
A. Crosiema	Hungary	32.4	1.86	5.40	1:8
Smena	USSR (Far East)	29.0	1.74	6.00	1:8
Chernovitskaya 5	Uk SSR	31.3	1.40	4.47	1:16
Amurskaya 401	USSR (Far East)	33.5	1.30	3.88	1:32
Amurskaya 400	USSR (Far East)	30.9	1.20	3.88	1:64
Amurskaya 266	USSR (Far East)	32.8	1.44	4.39	1:16
Dun-nun	China	29.6	2.16	7.30	1:4
Palmetto	USA	29.7	2.22	7.47	1:8
Normann	USA	30.0	1.40	4.67	1:8
Charly	USA	31.3	1.06	3.38	1:4
Morsoy	USA	28.4	2.00	7.04	1:2
Rampage	USA	30.0	1.10	3.67	1:8
VIR-6385	Australia	30.0	1.24	4.13	1:32

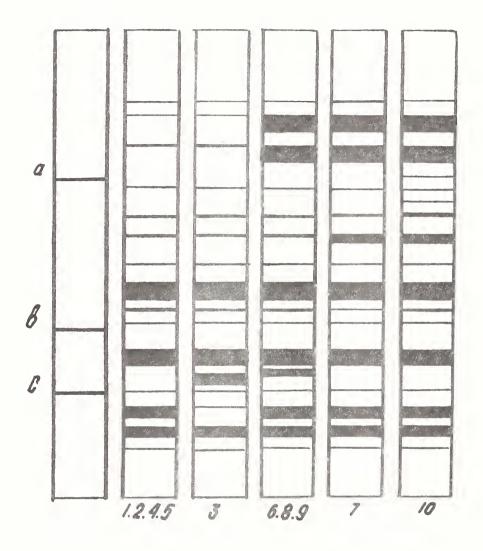


Figure 1. The electrophoregram of agglutinate active protein fractions

- 1 Pavlikeni
- 2 Rouest
- 3 Carona
- 4 Smena
- 5 Pshebedonska
- 6 Zora
- 7 Heimkraft
- 8 Dun-nun
- 9 A. Crosiema 10 - Palmetto
- a- bovine serum albumin (68000)
- b- polyedren protein (28600)
- c- RNA-ase (14000)

distribution of their components. Total and specific protein fractions were pointed out for different soybean cultivars. On the basis of the content of fast- and slow-moving components, it became possible to determine two general varietal groups. In the varieties 1-5 (all European origin) wide intensive dyed stripes were absent in zone of slow-moving fractions with maximum molecular weight. The varieties 6-10 (some European, American, and Chinese cultivars) had the clearest sign of these components. The varieties 7 and 10 had an additional stripe in this zone. There are some differences between varieties in zone of fast-moving components. Molecular weight of the fractions was 10000-85000 dalton.

Out data show that soybean varieties of various origin have essential differences in lectin content. Undoubtedly, ability to synthesize group-specific lectins is controlled genetically (Orf et al., 1978). Specification of varieties confirms the prospectivity of long-term breeding programs, when planning the creation of forms with valuable highly assimilated protein. Absence of correlation between total protein content and that of lectins give us an opportunity to assume possibility of soybean breeding for high protein content and optimal content of lectins. The breeding for protein quality, high yield, and environmental resistance requires subsequent study of genetics, physical and chemical qualities of lectins and their role in life of plants. The specific activity of lectins must be studied in accordance with human or animal organism. The breeding of legume crops for protein quality requires the estimation of material on lectins presence.

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1) Contributory applied research in growing soybean in the Mekong Delta - Socialist Republic of Vietnam.

During the period from 1976 through 1982, several experiments were carried out by the Soybean Research Center, University of Cantho, Socialist Republic of Vietnam to find out the most suitable varieties and the best cultural practices in the area. Among these experiments were the following: varietal selection and evaluation, interaction of agronomical physiological characteristics and tendency to boost seed yield in soybean, fertilization, seeding rate, row spacing, number of plants per hill, water requirements, and so on.

This communication is focused only on a collection of up-to-date experimental data available.

Varietal selection and evaluation: Four different soybean collections have been tested in 18 experiments with the unique procedure of randomized complete block design (RCBD) or sub-plot design (SBD) with 3 or 4 replicates.

With four soybean collections, it was found that, in soybean variety DH4, although its seed yield per hectare was not so high, its growth duration was shorter (75 days), and its productive efficiency was rather high; i.e., seed yield was approximately 21 kg/ha/day. The other soybean varieties, MTD 10 (a pedigree of 'Santa Maria'/'Wisconsin CF 111', a cross performed at the Soybean Research Center in 1974), and MTD 13 (an exotic soybean variety newly introduced from the Philippines in 1979) always showed a good yield performance in all experiments, especially in dry season. Seed yield of MTD 13 averaged 1.95 tons/ha. Their growth duration was the same - 85 days. In addition, there were two soybean entries, MTD 22 and MTD 65, that gave a potential seed yield rather high, 2.46 tons/ha and 2.19 tons/ha, respectively, but both their growth duration was longer - 91 days.

Interaction of some agronomic characters in three promising soybean varieties, MTD 10, MTD 13 and DH4: Based on experimental data of days to bloom, growth duration, 100-seed yield, number of branches, number of internodes, number of pods per plant, number of seed per square meter, and seed-yield efficiency, it was shown that, in MTD 10 and MTD 13, there was no statistically significant difference; in DH4 soybean variety, there was a statistically significant difference. In three soybean varieties, it was found that days to bloom, growth duration, number of internodes, number of pods per plant, 100-seed yield, and number of seed per square meter were all significantly different. But plant height and seed-yield efficiency of two soybean varieties, MTD 10 and MTD 13, were not significantly different, while number of internodes and seed-yield efficiency were not significantly different in MTD 10 and DH4.

In considering the influence of nine agronomic characters on soybean seed yield, it was found that, in three soybean varieties tested, number of seed per square meter seemed to be closely related to seed yield, while number of pods per plant seemed to have a little relation to seed yield. Besides, seed

yield efficiency affected the seed yield of MTD 13. One-hundred-seed weight, and number of internodes significantly exerted a positive effect on seed yield, and days to bloom had a negative relation to seed yield. In DH4 soybean variety, we should also consider two characters, plant height and 100-seed yield.

Influence of seeding rate, row spacing, and number of plants per hill on different soybean varieties: Four promising soybean varieties entered the experiment in different locations: Long khanh, Long an, Tien giang, Ben tre, An giang, and Hau giang. From these experiments, conclusions were made as follows:

Growth duration, interval of time of blooming, and time of pod formation were not influenced by seeding rate, number of plants per hill and row spacing, but they were especially influenced by characters of individual variety and time of planting.

Plant height had a tendency to increase with seeding rate. In the same seeding rate plant height seemed to be lower while number of plants per hill was higher and row spacing was wider.

One-hundred-seed yield and seed yield efficiency were not influenced by seeding rate, number of plants per hill, and row spacing.

Number of branches was rapidly reduced by higher seeding rate and by larger row spacing, but it was not changed in view of number of plants per hill.

Seed yield seemed to be proportional with seeding rate and in narrow row spacing, while number of seed per square meter had an effect on total seed yield. Number of seed per square meter increased with seeding rate in low number of plants per hill and in narrow row spacing.

With plant population of 500,000 per hectare, four soybean varieties (MTD 6, MTD 10, MTD 13 and DH 4) gave a high seed yield. These less branching soybean varieties DH4 and MTD 13 might be grown in higher seeding rate.

With row spacing 30-40 cm, we got the highest seed yield. This was perhaps due to shading effect of the canopy, a limiting factor of growth of weed.

Intercropping soybean with corn: Intercropping had no effect on plant height and growth duration of either corn or soybean. In intercropping corn with soybean at different seeding rates and varieties, total seed yield computed in rice tonnage (in proportion of 1 kg soybean equivalent to 4 kg corn equivalent to 4 kg rice) was higher in monoculture of corn and in that of DH4 soybean variety. It was lower in monoculture of MTD 13 in rainy season, but this fact still depends on current price of individual crop tested. Soybean variety MTD 13 seemed to be the most suitable one in mixed culture with corn. In intercropping, the multiple cropping index was higher. In order to have the same production of seed of either corn or soybean, cultivated land acreage must be higher from 21 % in rainy season to 43% in dry season.

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Technical papers presented at a science and technology conference held at the University of Cantho in 1982 (in mimeograph).

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### 1) Summary of National Soybean Variety Test (1982/83)

Results of the National Soybean Variety Trial are reported in Tables 1 and 2. Sixteen entries, comprising seven recommended varieties and nine new promising cultivars, were tested at seven different locations.

Though the season started favorably, rains in December, 1982, were inadequate in the southern half of the country for good stand establishment. Long drought spell towards the end of the season occurred in most parts of the country, which affected soybean yield with the exception of Magoye where only about 500 mm of rain fell from December, 1982, to April, 1983. Distribution of rain was more of a problem than the total rainfall. The experiments suffered as no supplemental irrigation was available at any location except Mpongwe where the highest yield was obtained.

Recommended cultural practices using conventional tillage operations were used in establishing the tests at each location. The inter-row spacing was 50 cm and 200 seeds/5 m row were planted at a depth of about 3 cm. Herbicides such as Dual, Lasso, or Treflan and in some cases Sencor, were used at recommended rates. No disease control was necessary. Yield data were determined by harvesting two central 5-meter rows of each entry.

Mean yield data for seven locations are given in Table 1. The highest soybean yields were produced at Mpongwe followed by Mufulira. The lowest soybean yield was obtained at Luapula.

Cultivar 'Santa Rosa' gave the highest yield, followed by 'Kaleya', 277/6/10 ('Duiker') and 'Tunia' (Table 2).

At Magoye (Southern Province), the yield response of different cultivars was significant at 1% level. Though the highest yielding cultivar was Kaleya, there were no significant differences in yield between Kaleya, Santa Rosa, 278/5/5, 277/6/10 (Duiker), 'Sable', 'Hale 3', 'Magoye', and Tunia (Table 1). The yield was generally depressed due to low rainfall and two long dry spells from February 15, 1983 to March 4, 1983 and March 26, 1983 to April 25, 1983.

At Golden Valley (Central Province), the yield response of cultivars was significantly different at 5% level. The highest yield was obtained from Kaleya but this was not significantly different from 'Geduld', Tunia, Magoye, 'Kudu', Santa Rosa and Hale 3 (Table 1).

At Kabwe (Central Province), there was no significant difference in the yield response of different cultivars; however, the top three varieties were Santa Rosa, Tunia and Kaleya.

At Mufulira (Copperbelt Province), the yield response of cultivars was highly significant (0.1% level). Kudu gave the highest yield but there was no significant difference in the yield between Kudu, 'Jupiter', Santa Rosa, 278/5/5/, 277/5/6, 277/6/10 (Duiker) and 'Hernon 147' at 5% level.

At Mpongwe (Copperbelt Province), highest yield was obtained from 277/6/10 (Duiker) which produced 3940 kg/ha; however, the difference in yield was not statistically significant between 277/6/10 (Duiker), Sable, and 199/6/40 at 5% level.

Table 1. Yield of soybean varieties at different locations

	Locations						
Cultivar	Magoye	Golden Valley	Kabwe	Mufulira	Mpongwe	Msekera	Luapula
	kg/ha						
Geduld	1144	1764	1814	1850	2600	1202	1276
Hernon 147	966	1398	2018	2316	2910	910	610
Hale 3	1352	1524	1704	1910	3080	1322	1228
Magoye	1280	1752	1616	2176	2390	1198	1052
Kaleya	1704	1850	2182	2836	3390	998	700
Santa Rosa	1476	1692	3128	2646	2800	1018	1558
Jupiter	1042	1172	1232	2676	2590	672	908
Tunia	1256	1756	2412	2280	3270	932	872
278/5/5	1458	1048	1474	2370	3010	1136	942
Kudu	890	1736	1342	2856	3310	1196	910
Sable	1410	950	1988	2256	3430	1090	370
199/6/40	812	1228	926	1246	3430	1232	1330
P221/6/28	992	828	1994	1496	2800	1556	1220
277/6/10 (Duiker)	1430	1216	2090	2320	3940	1502	806
P277/5/6	894	1100	1710	2356	3210	1256	566
P221/7/10	1198	1154	1482	2170	3350	1158	920
Mean	1207	1385	1820	2235	3076	1149	954
Soil type	Sandy 1oam	Sandy çlay loam	Sandy loam	Sandy loam	Sandy clay loam	Sandy clay loam	Sandy loam
LSD 5%*	456.9	721.0	N.S.	559.4	524.4	388.2	N.S.

Table 2. Mean yield and percent control of different varieties

Rank	Cultivar	Yield/ha	% control <sup>a</sup> 82/83	% control 81/82	% control 80/81
		kg			
1	Santa Rosa	2045	112	107	114
2	Kaleya	1950	107	109	118
3	277/6/10 (Duiker)	1901	104	-	-
4	Tunia	1825	100	105	106
5	Kudu	1749	96	94	-
6	Hale 3	1731	95	81	104
7	P211/7/10	1713	94	-	-
8	Geduld	1664	91	96	99
9	Sable	1642	90	98	-
10	Magoye	1638	90	92	111
11	278/5/5	1634	90	-	-
12	Hernon 147	1590	87	96	115
13	P277/5/6	1585	87	-	-
14	P221/6/28	1555	85	82	-
15	Jupiter	1470	81	84	69
16	199/6/40	1458	80	90	-

<sup>&</sup>lt;sup>a</sup>Control = Mean of Santa Rosa + Kaleya + Jupiter = 1822 kg/ha.

At Msekera (Eastern Province), the yield response of cultivars was significant at 1% level. The highest yield was obtained from P221/6/28 but this yield was not significantly different at 5% level from 277/6/10 (Duiker), Hale 3, P277/5/6, 199/6/40, Geduld, Magoye, and Kudu. Msekera received 1000.9 mm rainfall which was 33.1 mm less than the normal. There was also a dry spell from February 17, 1983, to March 1, 1983.

At Mansa (Luapula Province), differences in yield response of cultivars were not statistically significant. However, Santa Rosa gave the highest yield, followed by 199/6/40 and Geduld (Table 1).

Yield response of 16 cultivars, percent control 82/83, 81/82, 80/81 and mean percent control are given in Table 2 ( $\bar{x}$  of 7 locations). Santa Rosa, Kaleya, and Tunia have done well consistently for three years as compared to control. Control is the average yield of Santa Rosa, Kaleya, and Jupiter. Cultivar 277/6/10 (Duiker) was not tested in previous years but during 82/83 it gave 4% more yield than the control and was third in the overall position (Table 2).

Based on the performance in this and the past years, the following varieties are recommended: Santa Rosa, Hernon 147, Kaleya, Magoye, and Jupiter.

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# VI. INDEX OF AUTHORS

41 1 0	~ 1 1 D D	6.1
Ahmed, S	Igbokwe, P. E	61
Allen, J. R 78	Javaheri, F	
Atherly, A. G 100	Joshi, J	70
Broich, S. L 97	Joshi, J. M	
Banga, S. K 42,45	Juvick, G. A	15
Banga, S. S 42,45	Kakati, N. M	34
Benepal, P. S 63	Kalita, M. M	29,34
Bernard, R. L 15	Kerketta, B	43
Bhagsari, D. S 70	Kiang, Y. T	135,140,143
Bowers, G. R., Jr 150	Konwar, B. K	38
Broich, S. L 97	Marjushkin, V. F	163
Brown, A. H. D 17	Matsumoto, S	49
Brown, J 132	Mookherji, S	76
Burdon, J. J 17	Murphy, L	70
Burton, J. W 146	Nelson, R. L	14,15
Carter, T. E., Jr 146	Newhouse, K. E	105
Chernaja, I. V 163	Nkumbula, S	171
Chiang, Y. C 135,140,143	Pacumbaba, R. P	63
Choudhury, A. K 21,24,27	Palmer, R. G	9,91,92,94,97,
Choudhury, A. K 21,24,27  Dadson, R. B 70	Palmer, R. G	100,102,105,111,
Dadson, R. B 70		100,102,105,111, 115,127
Dadson, R. B 70 Delannay, X 92,97	Pathak, A. K	100,102,105,111, 115,127
Dadson, R. B		100,102,105,111, 115,127
Dadson, R. B 70 Delannay, X 92,97	Pathak, A. K	100,102,105,111, 115,127
Dadson, R. B	Pathak, A. K Prakash, R	100,102,105,111, 115,127 31 42,43
Dadson, R. B.       70         Delannay, X.       92,97         Edung, S.       61         Erickson, E. H.       152         Fletcher, J.       83	Pathak, A. K  Prakash, R  Pushpendra	100,102,105,111, 115,127 31 42,43 46
Dadson, R. B.       70         Delannay, X.       92,97         Edung, S.       61         Erickson, E. H.       152         Fletcher, J.       83         Floyd, M.       76	Pathak, A. K  Prakash, R  Pushpendra  Ram, H. H	100,102,105,111, 115,127 31 42,43 46
Dadson, R. B.       70         Delannay, X.       92,97         Edung, S.       61         Erickson, E. H.       152         Fletcher, J.       83         Floyd, M.       76         Furuya, T.       49	Pathak, A. K.  Prakash, R.  Pushpendra  Ram, H. H.  Rangappa, M.	100,102,105,111, 115,127 31 42,43 46 46
Dadson, R. B.       70         Delannay, X.       92,97         Edung, S.       61         Erickson, E. H.       152         Fletcher, J.       83         Floyd, M.       76         Furuya, T.       49         Garner, R.       76         Golinskaya, E. L.       163	Pathak, A. K Prakash, R Pushpendra Ram, H. H Rangappa, M Reddy, M. R	100,102,105,111, 115,127 31 42,43 46 46 63
Dadson, R. B.       70         Delannay, X.       92,97         Edung, S.       61         Erickson, E. H.       152         Fletcher, J.       83         Floyd, M.       76         Furuya, T.       49         Garner, R.       76         Golinskaya, E. L.       163         Goodman, R. M.       83	Pathak, A. K Prakash, R Pushpendra Ram, H. H Rangappa, M Reddy, M. R Rupert, E. A	100,102,105,111, 115,127 31 42,43 46 46 63 77
Dadson, R. B.       70         Delannay, X.       92,97         Edung, S.       61         Erickson, E. H.       152         Fletcher, J.       83         Floyd, M.       76         Furuya, T.       49         Garner, R.       76         Golinskaya, E. L.       163         Goodman, R. M.       83         Gorman, M. B.       135,140,143	Pathak, A. K.  Prakash, R.  Pushpendra  Ram, H. H.  Rangappa, M.  Reddy, M. R.  Rupert, E. A.  Russel, L.	100,102,105,111, 115,127 31 42,43 46 46 63 77 80 61 63,76
Dadson, R. B.       70         Delannay, X.       92,97         Edung, S.       61         Erickson, E. H.       152         Fletcher, J.       83         Floyd, M.       76         Furuya, T.       49         Garner, R.       76         Golinskaya, E. L.       163         Goodman, R. M.       83         Gorman, M. B.       135,140,143         Grant, J. E.       17	Pathak, A. K.  Prakash, R.  Pushpendra  Ram, H. H.  Rangappa, M.  Reddy, M. R.  Rupert, E. A.  Russel, L.  Sapra, V. T.	100,102,105,111, 115,127 31 42,43 46 46 63 77 80 61 63,76
Dadson, R. B.       70         Delannay, X.       92,97         Edung, S.       61         Erickson, E. H.       152         Fletcher, J.       83         Floyd, M.       76         Furuya, T.       49         Garner, R.       76         Golinskaya, E. L.       163         Goodman, R. M.       83         Gorman, M. B.       135,140,143         Grant, J. E.       17         Grace, J. P.       17	Pathak, A. K.  Prakash, R.  Pushpendra  Ram, H. H.  Rangappa, M.  Reddy, M. R.  Rupert, E. A.  Russel, L.  Sapra, V. T.  Sarmah, S. C.	100,102,105,111, 115,127 31 42,43 46 46 63 77 80 61 63,76 21,24,27,29,31,34
Dadson, R. B.       70         Delannay, X.       92,97         Edung, S.       61         Erickson, E. H.       152         Fletcher, J.       83         Floyd, M.       76         Furuya, T.       49         Garner, R.       76         Golinskaya, E. L.       163         Goodman, R. M.       83         Gorman, M. B.       135,140,143         Grant, J. E.       17         Grace, J. P.       17         Graybosch, R. A.       102	Pathak, A. K.  Prakash, R.  Pushpendra  Ram, H. H.  Rangappa, M.  Reddy, M. R.  Rupert, E. A.  Russel, L.  Sapra, V. T.  Sarmah, S. C.  Shipe, E. R.	100,102,105,111, 115,127  31  42,43  46  46  63  77  80  61  63,76  21,24,27,29,31,34  80  100,111
Dadson, R. B.       70         Delannay, X.       92,97         Edung, S.       61         Erickson, E. H.       152         Fletcher, J.       83         Floyd, M.       76         Furuya, T.       49         Garner, R.       76         Golinskaya, E. L.       163         Goodman, R. M.       83         Gorman, M. B.       135,140,143         Grant, J. E.       17         Grace, J. P.       17         Graybosch, R. A.       102         Griffin, J. D.       91	Pathak, A. K.  Prakash, R.  Pushpendra  Ram, H. H.  Rangappa, M.  Reddy, M. R.  Rupert, E. A.  Russel, L.  Sapra, V. T.  Sarmah, S. C.  Shipe, E. R.  Shoemaker, R. C.	100,102,105,111, 115,127  31  42,43  46  46  63  77  80  61  63,76  21,24,27,29,31,34  80  100,111
Dadson, R. B.       70         Delannay, X.       92,97         Edung, S.       61         Erickson, E. H.       152         Fletcher, J.       83         Floyd, M.       76         Furuya, T.       49         Garner, R.       76         Golinskaya, E. L.       163         Goodman, R. M.       83         Gorman, M. B.       135,140,143         Grant, J. E.       17         Grace, J. P.       17         Graybosch, R. A.       102         Griffin, J. D.       91         Haque, M. F.       43	Pathak, A. K.  Prakash, R.  Pushpendra  Ram, H. H.  Rangappa, M.  Reddy, M. R.  Rupert, E. A.  Russel, L.  Sapra, V. T.  Sarmah, S. C.  Shipe, E. R.  Shoemaker, R. C.  Sichkar, V. I.	100,102,105,111, 115,127  31  42,43  46  46  46  63  77  80  61  63,76  21,24,27,29,31,34  80  100,111  163
Dadson, R. B.       70         Delannay, X.       92,97         Edung, S.       61         Erickson, E. H.       152         Fletcher, J.       83         Floyd, M.       76         Furuya, T.       49         Garner, R.       76         Golinskaya, E. L.       163         Goodman, R. M.       83         Gorman, M. B.       135,140,143         Grant, J. E.       17         Grace, J. P.       17         Graybosch, R. A.       102         Griffin, J. D.       91	Pathak, A. K.  Prakash, R.  Pushpendra  Ram, H. H.  Rangappa, M.  Reddy, M. R.  Rupert, E. A.  Russel, L.  Sapra, V. T.  Sarmah, S. C.  Shipe, E. R.  Shoemaker, R. C.  Sichkar, V. I.  Silvoy, J. J.	100,102,105,111, 115,127  31 42,43 46 46 46 63 77 80 61 63,76 21,24,27,29,31,34 80 100,111 163 80

Singh, R. J	86,90	Tomar, J. B	42
Skorupska, H	53	Treick, R	132
Srivastava, M	45	Trivedi, H. B. P	43
Talukdar, P	30	Verma, V. D	46
Tin, C. H	169	Wilson, K	132
Tiwari, C. C	61	Wongyai, W	49
		Yee, C. C	115

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